The 57th Annual Meeting of the International Association of Forensic Toxicologists.

2nd - 6th September 2019
BIRMINGHAM, UK
The ICC
Birmingham Broad Street, Birmingham B1 2EA

Programme & Abstracts
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### Oral Programme

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Welcome Message

It is our great pleasure to welcome you to the UK for the 57th Annual Meeting of The International Association of Forensic Toxicologists (TIAFT) between 2nd and 6th September 2019.

It has been decades since the Annual Meeting has taken place in the country where TIAFT was founded over 50 years ago. The meeting is supported by LTG (London Toxicology Group) and the UKIAFT (UK & Ireland Association of Forensic Toxicologists) and we thank all our exhibitors and sponsors. Please visit them in the exhibit hall and at the vendor seminars taking place Tuesday to Thursday.

The venue for the meeting is the renowned International Convention Centre (ICC) in Birmingham in the heart of England. Birmingham is a large and diverse city full of heritage with modern developments. The Welcome Reception is at Birmingham Town Hall, with the traditional cultural trip on Wednesday afternoon visiting Shakespeare’s birthplace and home town of Stratford-Upon-Avon as well as an evening medieval experience at Warwick Castle. After the TIAFT Business meeting, the conference finishes with the TIAFT Gala Dinner at the ICC on Friday evening.

On the accompanying pages you will see a strong scientific agenda relevant to modern toxicology and we thank all those who submitted an abstract and the Scientific Committees for making the scientific programme a success. Starting with a large Young Scientists Symposium and Dr Yoo Memorial plenary lecture by Prof Tony Moffat on Monday, there are oral session topics in Clinical & Post-Mortem Toxicology on Tuesday, Human Behaviour Toxicology & Drug-Facilitated Crime on Thursday and Toxicology in Sport, New Innovations and Novel Research & Employment/Occupational Toxicology on Friday. In addition to this are the innovative e-posters in all topics available through the conference App and in the e-poster areas in the exhibit hall.

With over 700 delegates attending across the week, TIAFT2019 is an opportunity to learn, see old friends and make new ones as is the spirit of TIAFT. Enjoy the science, the city and the UK. TIAFT has come home.

Dr Simon Elliott
Chair and on behalf of the TIAFT2019 Organising Committee
Committees

TIAFT 2019 Organising Committee

Simon Elliott ........................... Chair
Peter Streete ......................... Finance Lead
Susie Davies ......................... Social Programme Lead
Peter Maskell & Jarrad Wagner  Scientific and Exhibitor Leads
Sue Paterson .......................... Chair
Jenny Button ......................... Social Media Lead
John Farina ........................... Legal

International Scientific Committee

Serap Akgur ......................... Turkey
Sotiris Athanaselis ............... Greece
Volker Auwärter .................... Germany
Patrick Best ......................... Barbados
Wim Best ............................. Netherlands
Jochen Beyer ......................... Switzerland
Federica Bortolotti ................. Italy
Craig Chatterton ................. Canada
Gail Cooper .......................... USA
Marc Devaux ......................... France
Luis Ferrari .......................... Argentina
Dimitri Gerostamoulos ........... Australia
Teemu Gunnar ....................... Finland
Marilyn Huestis ...................... USA
Wayne Jones ......................... Sweden
Pascal Kintz ......................... France
Robert Kronstrand ................. Sweden
Raphael Lanaro ..................... Brazil
Sooyeun Lee ......................... Korea
Barry Logan ......................... USA
Markus Meyer ....................... Germany
Luca Morini ......................... Italy
Frank Peters ......................... Germany
Sumandeep Rana .................... USA
Luke Rodda ......................... USA
Mark Stephenson ................. Australia
Christophe Stove ................. Belgium
Helena Teixeira ................. Portugal
Jarrad Wagner ...................... USA
Chip Walls ........................... USA
Wolfgang Weinmann ............. Switzerland
Sarah Wille ......................... Belgium
Yi Ju Yao ............................. Singapore
Dariusz Zuba ......................... Poland

Additional thanks and support;

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Vice-Chair ................................ Atholl Johnston
Past-Chair ................................ Sue Paterson
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Treasurer/Webmaster ............... Peter Streete
Council Members ..................... Susan Grosse
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                                      Yvonne Kavanagh
                                      John Farina
                                      Sunella Brahma
                                      Mike Scott-Ham

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Treasurer ................................ Atholl Johnston
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Committee Members .................. Alan Brailsford
                                      Lewis Couchman
                                      Jeffery Hackett
                                      Alex Lawson
                                      Jay Schlesinger
                                      Paul Smith
                                      Lorna Nisbett

UK Scientific Committee

Rebecca Andrews ................... Limon Nahar
Simon Cosbey ......................... David Osselton
David Cowan .......................... Mark Parkin
Julie Evans .......................... Collin Seneviratne
Bob Flanagan ......................... Paul Smith
Steve George ......................... Duncan Stephen
Susan Grosse ......................... Kerry Taylor
Atholl Johnson ...................... Hazel Torrance
Nick Lemos .......................... Kirsten Turner
Peter Maskell
General Information

Abstracts
This abstract book is produced as an e-book only – there is no printed edition. Abstracts can also be viewed in the conference App via a smartphone, tablet or at one of the e-screen areas. Abstract details, including authors and affiliations have been reproduced as submitted.

Catering and Dietary requirements
All morning, lunch and afternoon breaks will be provided in the exhibition hall area (ICC Hall 3), except for Friday. For timings please refer to the conference schedule. If you have advised of special dietary requirements during online registration, this has been catered for as appropriate. Please speak to a member of catering staff at the commencement of each meal break or social function.

Conference Venue
The International Convention Centre (ICC)
The ICC Birmingham, Broad Street, Birmingham B1 2EA, UK.

Conference App
The conference App (available for iOS and Android) can be found using “TIAFT2019” for download. The schedule, scientific programme, abstracts, e-posters and more can be accessed by delegates.

Emergency Details
In an emergency, telephone 999 for Ambulance, Fire Service or Police and/or seek immediate help.

Exhibition Hall (Hall 3) Opening Times
The exhibition hall (ICC Hall 3) will be open at the following times.

- Monday 2nd September: 18:00 – 19:00
- Tuesday 3rd September: 08:00 – 18:00
- Wednesday 4th September: 08:00 – 12:30
- Thursday 5th September: 08:00 – 18:00
- Friday 6th September: Closed

Internet and WIFI Access
Wireless internet (Wi-Fi) will be available free of charge for delegates at the ICC.

Mobile Phones and Electronic Devices
Whilst you can use your mobile phones and electronic devices to access the conference App and e-book during sessions, as a courtesy to speakers and your fellow delegates, please turn phones and devices to “silent” during presentations and whilst in session.

Name Badges and Lanyards
For security purposes, delegates, speakers, exhibitors and guests are required to wear their name badge to all sessions, the exhibition and social functions. Badges may be scanned. Entrance into sessions and social events are restricted to appropriately registered persons only. If you misplace your name badge please see staff at the registration desk to arrange a replacement.

Notepad and Pen
Every registered delegate will receive a note pad and pen in their conference bag.

Oral Programme
Every endeavour has been made to produce an accurate programme. If you are presenting at the conference, please ensure you are aware of your presentation time as contained within the programme in this conference e-book and in the App. The overall conference schedule and oral programme is also available as a printed schedule. The organisers reserve the right to change the conference programme at any time without notice.

Posters
There are no traditional poster sessions at TIAFT2019 whereby authors should be at their poster at a given date or time. All authors of posters have been asked to upload their poster as a portrait pdf for viewing within the conference App, either on a smartphone, tablet or at one of the e-screen areas. Authors are responsible to ensure their poster(s) have been provided and in the correct format. The conference organisers are not responsible for the poster content.

Attendees wanting to discuss a poster can contact the author through the conference App. Posters can be presented at an e-screen area via the connected tablet with the conference App.

Young Scientists have been asked to also produce physical posters to be displayed throughout the conference at the designated posterboard area in the Exhibition Hall (ICC Hall 3). Authors have responsibility for this.

Registration and Information Desks
The registration and information desks are located on Level 1 of the ICC up the stairs/escalator from the main ground floor public concourse. The desks will be open from 8am throughout the conference, where possible. There is no registration on Wednesday 4th September.

Smoking
Smoking is not permitted indoors at the ICC.

Speakers
Please ensure you have loaded your oral presentation with the audio visual technicians in the Media Suite at least 3 hours prior to your scheduled presentation time. Each oral presentation is timetabled for 10 minutes with 2 minutes for questions and speakers should adhere to this timing otherwise presentations may have to be curtailed by the session Moderators.

Disclaimers
The conference organisers have made every attempt to ensure that all information in the programme/abstract e-book, conference App and printed schedule book is correct; it is also subject to change. Much of the information has been provided by external sources. The conference organisers cannot accept liability for personal injuries sustained, for loss of, or damage to, property belonging to delegates (or their accompanying persons), either during or as a result of the conference or associated tours/activities. Please check the validity of your own travel insurance.
The ICC and Symphony Hall Layout

Key

A  Box Office/Business Reception
B  Starbucks
C  WH Smiths
D  Oak Kitchen
E  THSH Shop
F  Castle Fine Art

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Accessible toilets

Key
- Staircase
- Escalator
- Lift
- Lift Route
- Toilet
- Baby Change
- Cloak Room
- Symphony Hall
- Box Office
- Eating & Drinking
- Prayer Room

Key - Accessible toilets

Level 2
- Lower Mall, Castle Fine Art (Key Symbol)
- Nest to Castle Fine Art Gallery
- Hall 9 & 10 Foyer

Level 3
- Box Office (key symbol)
- Symphony Hall Foyer
- Hall 7 Foyer
- Hall 11 Foyer
- Main Cloakroom (Key symbol)

Level 4
- Hall 7 Foyer
- Hall 1, Door 1
- Hall 3, Door 1

Level 5
- Hall 5 Foyer
- Hall 4 Foyer
- Executive Rooms

Level 5a
- Executive Rooms

Facilities marked with requires radar keys to be opened
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Exhibitor booths:

- Catering
- LCD Screens for e-posters (3 sites)
- Fixed posterboards for young scientists
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**Welcome Reception:**
**Monday 2nd September 2019:** 8-10pm

**Birmingham Town Hall**
Birmingham is the UK’s 2nd largest city located in the geographical heart of England and host city to the Commonwealth Games in 2022. It is a city full of heritage as well as modern developments, providing a fantastic venue for the event. The start of the conference week is celebrated with a Welcome Reception for full week delegates at Birmingham's Town Hall which is a very short walk from the ICC. Drinks and canape food will be provided along with an appearance by Birmingham historian, writer and broadcaster, Prof Carl Chinn MBE

**Delegate tour to Stratford-Upon-Avon and Warwick Castle:**
**Wednesday 4th September 2019:** 12:30-10:45pm

**Stratford-Upon-Avon**
After collecting lunch from the Exhibition Hall (ICC Hall 3), delegates will leave the ICC by coach. Stratford-upon-Avon is the starting location of the traditional TIAFT meeting excursion allowing informal interactions between delegates. The birthplace of William Shakespeare, Stratford-upon-Avon still contains the houses where Shakespeare was born and lived. As well as being the home to the Royal Shakespeare Company, the town is a popular tourist attraction. Delegates will travel by coach with a tour guide or freetime option and receive entry to a Shakespeare house (Birthplace or New Place town house).

**Warwick Castle**
Close to Stratford-upon-Avon, in the early evening, delegates will travel on to Warwick Castle. Originally established in 1068 by William the Conqueror, the stone castle was built in the 12th Century and is sure to impress both national and international visitors. Delegates will tour the castle grounds, with a jousting show followed by a medieval banquet and return to the ICC by coach.

**TIAFT Gala Dinner:**
**Friday 6th September 2019:** 8:00pm-12am

**The ICC, Birmingham**
The TIAFT Gala Dinner will take place in the transformed Hall 3 of the ICC for a sit-down 3 course meal with drinks and live band, including the prestigious TIAFT Awards and passing of the host flag.

*Over to you TIAFT2020 – Cape Town!*
It was with great sadness that TIAFT learned of the passing of Dr Youngchan Yoo in February 2019, husband of our current Past-President, Dr Heesun Chung. Dr. Yoo regularly attended TIAFT conferences and in 1990 was the first Korean forensic toxicologist to join TIAFT. He worked at Korea’s National Forensic Service (NFS) for 37 years where he became its 8th Director General. He was very skilled at instrument repairs – particularly their early gas chromatograph-mass spectrometers. When Korea began experiencing a problem with tetrodotoxin poisonings from consumption of puffer fish, Dr. Yoo developed a method to detect the toxin in blood and gastric contents.

Dr. Yoo knew of the importance of TIAFT to forensic toxicologists worldwide. He appreciated that continuous learning was a key to success in life. With this in mind, TIAFT has received a generous donation in the memory of Dr. Yoo for a plenary speaker at annual TIAFT meetings. It is our great pleasure to introduce the inaugural Youngchan Yoo Memorial Plenary Speaker, Prof Tony Moffat, a long-time TIAFT member and Alan Curry Awardee.

Tony graduated in pharmacy at Chelsea College, University of London and stayed on to do a PhD with Prof Arnold Beckett researching into the analysis of drugs in urine for anti-doping purposes. In 1970, after two years as Assistant Professor of Biochemistry at Baylor College of Medicine, Houston, Texas, he joined the Forensic Science Service (FSS) at its research laboratory in Aldermaston. He carried out research into leading methods for the analysis of drugs and poisons for forensic purposes and was later promoted to Head the Drugs and Toxicology Division. His work centred around developing methods that the operational laboratories of the FSS could use and his team developed new mass spectrometric, HPLC and immunological methods for their use. He also gave evidence in court on many notable cases.

In 1984, he went to the FSS Huntingdon Forensic Science Laboratory as its Assistant Director where his main responsibilities were to ensure the quality of the work of the laboratory, debriefing scene going forensic scientists and reviewing the scientists’ Statements of Witness in major cases. In 1989, he went to the Headquarters of the FSS in London as Head of Quality Management to assist the operational laboratories to get accreditation by the United Kingdom Accreditation Service to ISO 17025 as well as conforming to ISO 9000. He continued his operational experience when he returned to the Huntingdon Laboratory as its Resources Manager in 1990 until 1993 when he came back to research as the FSS Research Co-ordinator at the Birmingham Laboratory.

In 1994, he returned to pharmacy in the joint appointment of Chief Scientist at the Royal Pharmaceutical Society and Professor of Pharmaceutical Analysis at the UCL School of Pharmacy where he is still Emeritus Professor.

During his career, he has published over 370 publications, including nine books, the most important being his co-editorship of Clarke’s Analysis of Drugs and Poisons.

His awards include TIAFT’s Alan Curry award, British Pharmaceutical Conference Science Award, Society of Analytical Chemistry Silver Medal, Philip Allen Award of the Forensic Science Society, joint award of the BUCHI Award, the Academy of Pharmaceutical Sciences Medal and the Royal Pharmaceutical Society’s Charter Gold Medal.

The title of Prof Moffat’s plenary lecture is: “Things you should know and three proposals”.

Tony has asked that the content is to be a surprise but the purpose is to “to inform, educate and entertain”, which we are sure it will...
### Monday 2nd Sept
- **08:00 –** Registration
- **08:30 – 12:20** Executive Board Meeting and Young Scientists Symposium
- **12:20 – 13:20** LUNCH
- **13:20 – 14:20** Regional Representatives Meeting
- **14:20 – 16:30** Executive Board Meeting
- **16:30 – 18:00** Opening Ceremony
- **18:00 – 19:00** Exhibitors Hall Open
- **20:00 – 22:00** Welcome Reception

### Tuesday 3rd Sept
- **08:00 –** Registration
- **08:30 – 10:20** Post-Mortem Toxicology I
- **10:20 – 10:50** MORNING BREAK (Exhibition Hall)
- **10:50 – 12:20** Clinical Toxicology I
- **12:20 – 13:20** LUNCH (Exhibition Hall)
- **13:20 – 14:50** Post-Mortem Toxicology II
- **14:50 – 15:20** AFTERNOON BREAK (Exhibition Hall)
- **15:20 – 17:20** Clinical Toxicology II
- **17:45 – 19:15** Vendor Seminars

### Wednesday 4th Sept
- **08:30 – 10:00** Vendor Seminars (+ Exhibition Hall open)
- **10:20 – 11:50** Vendor Seminars (+ Exhibition Hall open)
- **12:00 – 12:30** LUNCH collection (Exhibition Hall)
- **12:30 – 17:30** Excursion to Stratford
- **17:30 – 22:45** Warwick Castle event + Banquet

### Thursday 5th Sept
- **08:00 –** Registration
- **08:30 – 10:20** Human Behavioural Toxicology (inc Driving) I
- **10:20 – 10:50** MORNING BREAK (Exhibition Hall)
- **10:50 – 12:20** Drug Facilitated Crime (inc DFSA) I
- **12:20 – 13:20** LUNCH (Exhibition Hall)
- **13:20 – 14:50** Human Behavioural Toxicology (inc Driving) II
- **14:50 – 15:20** AFTERNOON BREAK (Exhibition Hall)
- **15:20 – 17:20** Drug Facilitated Crime (inc DFSA) II
- **17:45 – 19:15** Vendor Seminars (Exhibition Hall closes)

### Friday 6th Sept
- **08:00 –** Registration
- **08:30 – 10:20** Toxicology in Sport
- **10:20 – 10:50** MORNING BREAK
- **10:50 – 12:20** New Innovations and Novel Research in Toxicology I
- **12:20 – 13:20** LUNCH
- **13:20 – 14:50** Employment/Occupational Toxicology
- **14:50 – 15:20** AFTERNOON BREAK
- **15:20 – 16:20** New Innovations and Novel Research in Toxicology II
- **16:30 – 18:30** TIAFT Business Meeting
- **20:00 – 00:00** Gala Dinner

*Times and items subject to any required changes.*
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Innovation with Integrity
YOUNG SCIENTISTS SYMPOSIUM
Oral Session

MONDAY 2nd September 08:30 - 13:20 (Hall 11)

09:00 - 09:20  Introduction

09:20 - 09:50  Mentor talk - Robert Kronstrand: “Do’s and don’ts on how to start up research projects/teams”

09:50 - 10:20  Caitlyn Norman: “Impact of NPS legislation on prevalence and how this has changed drug prevention?”

10:20 - 10:50  YS SYMPOSIUM BREAK

10:50 - 11:10  Marta Concheiro: “Improving Forensic Toxicology Education in the USA”.

11:10 - 11:30  Luke Rodda and Sarah Wille: “Basic principles of LEAN and examples for a Tox Lab”.

11:30 - 11:50  Carolina Noble (winner YS Latin America): “Bromo-dragonfly, a psychoactive benzodifuran resistant to hepatic metabolism and potently inhibits monoamine oxidase A”.

11:50 - 12:10  Shimpei Watanabe: winner YS presentation

12:20 - 13:20  LUNCH FOR YS SYMPOSIUM WITH TIAFT BOARD

OTHER PROCEEDINGS

MONDAY 2nd September 13:20 - 22:00

13:20 - 14:20  TIAFT Regional Representatives (Hall 11)

14:20 - 14:50  TIAFT Board

14:50 - 15:20  BREAK FOR TIAFT BOARD

15:20 - 16:20  TIAFT Board

16:30 - 18:00  OPENING CEREMONY (Hall 1)

EVENING
18:00 - 19:00  Exhibitor Hall Open (Hall 3)

20:00 - 22:00  Welcome Reception (Birmingham Town Hall)
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# POST-MORTEM TOXICOLOGY

## Oral Session I

**TUESDAY 3rd September 08:30 - 10:20 (Hall 1)**

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<th>Moderators</th>
<th>Abstract ID</th>
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<th>Authors</th>
<th>Institutions</th>
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<tr>
<td><strong>PM1</strong> 08:32</td>
<td>319</td>
<td>Investigation into the role of alcohol and cocaine in violent suicide.</td>
<td>Sue Paterson, Rebecca Andrews, Limon Nahar</td>
<td>Imperial College</td>
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<td><strong>PM2</strong> 08:44</td>
<td>183</td>
<td>Recognition of drugs showing exceptionally high or low median concentration levels in post-mortem femoral blood.</td>
<td>Ilkka Ojanperä, Pirkko Kriikku, Raimo A. Ketola</td>
<td>University of Helsinki and National Institute for Health and Welfare</td>
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<td><strong>PM3</strong> 08:56</td>
<td>193</td>
<td>Detection of New Psychoactive Substances (NPSs) in Dried Blood Spots (DBSs) collected from postmortem cases.</td>
<td>Luca Morini, Francesca Freni, Matteo Moretti, Claudia Vignali, Angelo Groppi</td>
<td>Department of Public Health, University of Pavia</td>
</tr>
<tr>
<td><strong>PM4</strong> 09:08</td>
<td>22</td>
<td>The difficult interpretation of toxicological analyses in formalin-fixed tissues: a case report involving oxycodone and midazolam.</td>
<td>Alice Ameline, Laurie Gheddar, Jean-sébastien Raul, Pascal Kintz</td>
<td>Institut of Legal Medicine, Strasbourg, France</td>
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<tr>
<td><strong>PM5</strong> 09:20</td>
<td>431</td>
<td>Death involving pong pong seed (Cerbera odollam)</td>
<td>Yi Ju Yao, Gina Chew</td>
<td>Clinical and Forensic Toxicology unit, Analytical Toxicology Laboratory, Health Sciences Authority</td>
</tr>
<tr>
<td><strong>PM6</strong> 09:32</td>
<td>354</td>
<td>Oxycodone findings and CYP2D6 function in post mortem cases.</td>
<td>Gerd Jakobsson</td>
<td>Department of Forensic Genetics and Forensic Toxicology, National Board of Forensic Medicine, Linköping, Sweden</td>
</tr>
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<td></td>
<td>Gerd Jakobsson¹, Lucia Pelle², Ronja Larsson¹, Henrik Gréen¹, Robert Kronstrand¹</td>
<td>¹Department of Forensic Genetics and Forensic Toxicology, National Board of Forensic Medicine, Linköping, Sweden, ²Division of Drug Research, Department of Medical and Health Sciences, Linköping University, Sweden</td>
</tr>
</tbody>
</table>
PM7  09:44  Abstract ID  361  
A UHPLC-MS/MS method for the detection of drugs and pharmaceuticals in human bones.  
Amvrosios Orfanidis  
Laboratory of Forensic Medicine & Toxicology, School of Medicine, Aristotle University Thessaloniki  
Amvrosios Orfanidis1, Helen Gika2, Georgios Theodoridis3, Orthodoxia Mastrogianni4, Eleni Zaggelidou1, Nikolaos Raikos2  
1Laboratory of Forensic Medicine & Toxicology, School of Medicine, Aristotle University Thessaloniki, 2Laboratory of Forensic Medicine & Toxicology, Medical School, Aristotle University Thessaloniki, 3School of Chemistry, Laboratory of Analytical Chemistry, Aristotle University Thessaloniki, 4Laboratory of Forensic Service of Ministry of Justice of Thessaloniki

PM8  09:56  Abstract ID  498  
Relationship between betahydroxybutyrate (BHB) concentrations in post-mortem blood and cause of death.  
Merete Vevelstad  
Oslo University Hospital  
Lena Midtlyng, Gudrun Høiseth, Lena Kristoffersen, Hege Luytkis, Maren Strand, Marianne Arnestad, Merete Vevelstad  
Oslo University Hospital

PM9  10:08  Abstract ID  62  
Katherine Wong  
Victorian Institute of Forensic Medicine  
Katherine Wong, Dimitri Gerostamoulos, Linda Glowacki, Alex Kotsos, Yeliena Baber  
Victorian Institute of Forensic Medicine

10:20  BREAK

CLINICAL TOXICOLOGY
Oral Session I
TUESDAY 3rd September 10:50 - 12:20 (Hall 1)

Moderators  Hans Maurer (Germany) & Bob Flanagan (UK)

CL1  10:56  Abstract ID  555  
Responding to patients and drug trends: advancement and development of clinical toxicology services at the National Rehabilitation Center, Abu Dhabi.  
H.E. Hamad Al Ghaferi  
National Rehabilitation Center, Abu Dhabi, UAE  
H.E. Hamad Al Ghaferi1, Abuelgasim Alhassan2, Samya Al Mamari3  
1National Rehabilitation Center, Abu Dhabi, UAE

CL2  11:08  Abstract ID  263  
All or Nothing Retrospective Evaluation of EtG Adherence Analysis Results in the Context of Organ Transplantation.  
Dirk K. Wissenbach  
Institute of Forensic Medicine, Jena  
Dirk K. Wissenbach1, Julia Dinger2, Daniela Remane2, Frank T. Peters2  
1Institute of Forensic Medicine, Jena, 2Institute of Forensic Medicine, Jena University Hospital, Jena, Germany
**CL3  11:20 Abstract ID 132**

Tobacco markers in meconium, maternal interview and neonatal outcomes.

**Ana de Castro**

University of Santiago de Compostela

Angela López-Rabuñal¹, Ana de Castro¹, Elena Lendoiro¹, Marta Concheiro-Guisán², Manuel López-Rivadulla¹, Angelines Cruz¹

¹University of Santiago de Compostela, ²John Jay College of Criminal Justice

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**CL4  11:32 Abstract ID 162**

Suspicion of GHB poisoning in a young child highlighted with a rapid enzymatic assay.

**Laurence Labat**

Assistance Publique Hôpitaux de Paris

Laurence LABAT¹, Pauline Thiebot®, Hervé Gourlain², Marion Soichot³, Marion LECLERCQ³, Brigitte DELHOTAL², Anne Laure Pelissier³

¹Assistance Publique Hôpitaux de Paris, ²Assistance Publique - Hôpitaux de Paris, ³Assistance Publique - Hôpitaux de Marseille

---

**CL5  11:44 Abstract ID 179**

Differences in metabolism of ATM4 between Asian and Caucasian subjects using street heroin.

**Pai-Shan Chen**

Department and Graduate Institute of Forensic Medicine

Pai-Shan Chen¹, Po-Hsin Kong¹, Da-Peng Yang¹, Tsang-Yaw Lin², Claire George³, Andrew Kicman⁴

¹Department and Graduate Institute of Forensic Medicine, ²Tsao-Tun Psychiatric Center, Ministry of Health and Welfare, Taiwan, ³Alere Toxicology, ⁴Drug Control Centre, Analytical & Environmental Sciences Division, King's College London

---

**CL6  11:56 Abstract ID 194**

A Simple Online Extraction LC/LC Atmospheric Pressure Ionization (APCI) MS/MS Assay for the Analysis of 17 Cannabinoids and Metabolites in Human Plasma.

**Cristina Sempio**

University of Colorado Anschutz Medical Campus

Cristina Sempio, Jelena Klawitter, Nohemi Almaraz-Quinones, Wanzhu Zhao, George Sam Wang, Kelly Knupp, Uwe Christians, Jost Klawitter

University of Colorado Anschutz Medical Campus

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**CL7  12:08 Abstract ID 236**

Analysis of Mushroom and Plant Toxins in Human Urine by Means of Normal Phase Liquid Chromatography Coupled to High Resolution Mass Spectrometry.

**Thomas P. Bambauer**

Department of Experimental and Clinical Toxicology, Institute of Experimental and Clinical Pharmacology and Toxicology, Center for Molecular Signaling (PZMS)

Thomas P. Bambauer¹, Lea Wagmann², Hans H. Maurer², Armin A. Weber², Markus R. Meyer²

¹Department of Experimental and Clinical Toxicology, Institute of Experimental and Clinical Pharmacology and Toxicology, Center for Molecular Signaling (PZMS), ²Department of Experimental and Clinical Toxicology, Institute of Experimental and Clinical Pharmacology and Toxicology, Center for Molecular Signaling (PZMS)
PM10  13:20  Abstract ID  87  
Can cannabis really kill you?  
Olaf Drummer  
Victorian Institute of Forensic Medicine  
Dimitri Gerostamoulos, Olaf Drummer, Noel Woodford  
Victorian Institute of Forensic Medicine

PM11  13:32  Abstract ID  270  
The Trouble with Kratom: Analytical and Interpretative Challenges involving Mitragynine.  
Barry Logan  
NMS Labs  
Barry Logan¹, Donna Papsun¹, Aya Chan-Hosokawa¹, Kristopher Graf¹, Justin Brower²  
¹NMS Labs, ²OCME North Carolina

PM12  13:44  Abstract ID  172  
Qualitative and Quantitative Analysis of Cyanide in Blood by Headspace Gas Chromatography - Mass Spectrometry.  
Hetti Thanthri Thanuja Priyadarshani  
Government Analyst’s Department  
Hetti Thanthri Thanuja Priyadarshani¹, D.A.S. Sakunthaladevi Tannakoon², K.r.r. Mahanama³  
¹Government Analyst’s Department, ²Government Analyst’s Department, ³Isuru Mawatha, Pelawatta, Battaramulla, Sri Lanka, ⁴University of Colombo, Sri Lanka

PM13  13:56  Abstract ID  320  
Baclofen: to screen or not to screen?  
Limon Nahar  
Imperial College London, Toxicology Unit  
Limon Nahar¹, Sue Paterson²  
¹Imperial College London, Toxicology Unit, ²Imperial College London

PM14  14:08  Abstract ID  534  
Development and validation of a method for quantification of 28 psychotropic drugs in post mortem blood samples by modified micro-QuEChERS and LC-MS/MS.  
Taís Rodrigues  
University of Campinas  
Taís Rodrigues¹, Damila R. Morais², Victor A. P. Gianvecchio³, Márcio H. Matsubara³, Ricardo L. Cunha⁴, José Luiz Costa⁵  
¹University of Campinas, ²Shimadzu of Brazil, ³Forensic Toxicology Laboratory, Institute of Legal Medicine, Sao Paulo State Police, ⁴Institute of Analysis and Forensic Research, Aracaju-SE, ⁵Faculty of Pharmaceutical Sciences, University of Campinas

PM15  14:20  Abstract ID  57  
Statistical validation of changes in the rate of acute fatal alcohol poisonings.  
Pirkko Kriikku  
National Institute for Health and Welfare (THL)  
Pirkko Kriikku¹, Ilkka Ojanperä²  
¹National Institute for Health and Welfare (THL), ²University of Helsinki, Department of Forensic Medicine
CLINICAL TOXICOLOGY
Oral Session II

TUESDAY 3rd September 15:20 - 17:20 (Hall 1)

Moderators
Duncan Stephen (UK) & Frank Peters (Germany)

CL8 15:20
Abstract ID 535
Variability in content and dissolution profiles of MDMA tablets collected in the UK between 2001 and 2018 – potential risk to users?
Lewis Couchman
Analytical Services International

Lewis Couchman1, Anca Frinculescu2, Catarina Sobriera3, Trevor Shine2, John Ramsay2, Max Hecht1, Karin Kipper1, David Holt1, Atholl Johnston1
1Analytical Services International, 2TICTAC Communications, 3Quenn Mary’s University of London

CL9 15:32
Abstract ID 279
Comparing the Diagnostic Value of PEth and CDT in 6705 patients: relation to Age and Sex.
Gudrun Høiseth
Oslo University Hospital

Alexander Årving1, Aleksandar Djordjevic2, Thor Hilberg2, Gudrun Høiseth1, Vigdis Vindenes1, Stig Tore Bogstrand1
1Oslo University Hospital, 2Furst Medical Laboratory

CL10 15:44
Abstract ID 367
Severe cholinergic syndrome after accidental poisoning with chlormequat solution filled in a soft drink bottle.
Daniela Remane
Institute of Forensic Medicine, Jena University Hospital

Daniela Remane1, Ingo Hartter2, Dirk K. Wissenbach2, Christian Rabe3, Florian Eyer2, Frank T. Peters3
1Institute of Forensic Medicine, Jena University Hospital, 2Division of Clinical Toxicology and Poison Control Centre Munich, Department of Internal Medicine II, School of Medicine, Technical University of Munich, Munich, Germany, 3Institute of Forensic Medicine, Jena University Hospital, Jena, Germany
**Hide and seek: overcoming the masking effect of opioid antagonists in activity-based screening tests.**

**Annelies Cannaert**
Ghent University - Laboratory of Toxicology

Annelies Cannaert1, Marie Deventer1, Melissa Fogarty2, Amanda LA Mohr2, Christophe P Stove1
1Ghent University - Laboratory of Toxicology, 2The Center for Forensic Science Research and Education

**Monitoring drugs causing serious intoxication in the community though Emergency Department Admission Blood Psychoactive testing-Protocols & initial findings.**

**Peter Stockham**
Forensic Science SA

Peter Stockham1, Peter Stockham2, Sam Alfred3, Emma Partridge3, Daniel Haustead3, Jake Mallon3, Hannah Green3, Andrew Camilleri2, Kerryn Mason3, April Rivers-Kennedy3, Chris Kostakis2
1Forensic Science SA, 2Forensic Science South Australia, 3Royal Adelaide Hospital, 4Flinders Medical Centre, 5Lyell McEwin Hospital, 6The University of Adelaide

**Development of a Liquid Chromatography Quadrupole Time of Flight Mass Spectrometry Method for the Screening of Antihypertensive Drugs in Urine.**

**Alex Lawson**
University Hospitals Birmingham NHS FT

Alex Lawson, Helen Wiggins, Indranil Dasgupta
University Hospitals Birmingham NHS FT

**New Psychoactive Substances Abuse Situation in Singapore.**

**Hooi Yan Moy**
Health Sciences Authority

Hooi Yan Moy1, Yi Ju Yao2, Ching Yee Fong3, Chi Pang Lui2
1Health Sciences Authority, 2Health Sciences Authority

**Systematic Investigation on Matrix Effects of Neuroleptics in Plasma and Whole Blood Samples Analyzed on Three Different Mass Spectrometers.**

**Julia Dinger**
University Hospital Jena, Institut of Forensic Medicine

Julia Dinger, Daniela Remane, Dirk K. Wissenbach, Frank T. Peters
University Hospital Jena, Institut of Forensic Medicine

**Oral fluid as an alternative matrix for therapeutic drug monitoring of immunosuppressants.**

**Elena Lendoiro**
University of Santiago de Compostela

Lucía Paniagua-González1, Elena Lendoiro1, Esteban Otero-Antón1, Manuel López-Rivadulla1, Angelines Cruz-Landeira1, Ana de Castro1
1University of Santiago de Compostela, 2Universitary Clinical Hospital of Santiago de Compostela

**Development of an untargeted metabolomics analysis of cerebrospinal fluid samples for clinical laboratories.**

**Jingya Yan**
Centre for Forensic Science, University of Technology Sydney, Ultimo, NSW, Australia

Jingya (Jinni) Yan1, Unnikrishnan Kuzhiumpambili2, Sushil Bandodkar3, Russell Dale3, Shanlin Fu1
1Centre for Forensic Science, University of Technology Sydney, Ultimo, NSW, Australia, 2Climate Change Cluster, University of Technology Sydney, Ultimo, NSW, Australia, 3The Children's Hospital at Westmead, Westmead, NSW, Australia
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### Abstracts

**HB1 08:32 Abstract ID 428**

**Title:** DUID Trends post-Cannabis Legalization and a Comprehensive Analytical Approach for DUID Casework.

**Authors:** Luke N. Rodda, San Francisco Office of the Chief Medical Examiner

- Luke N. Rodda, Alessandra M. Rivera, Megan Farley, Sue Pearring
  - San Francisco Office of the Chief Medical Examiner

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**HB2 08:44 Abstract ID 11**

**Title:** Concentrations of Amphetamine and Methamphetamine in Whole Blood Samples Obtained In Drugs and Driving Cases (OUI) in the Commonwealth of Massachusetts.

**Authors:** Jeffery Hackett, Private

- Jeffery Hackett, Albert Elian
  - 1Private, 2Massachusetts State Police Crime Laboratory

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**HB3 08:56 Abstract ID 124**

**Title:** Drug Related Deaths and Driving Under the Influence of Drugs - Narcotest: Important indicators in monitoring drug use in Cyprus.

**Authors:** Katerina Liveri, Department of Forensic Chemistry and Toxicology, Cyprus State General Laboratory

- Katerina Liveri, Christiana Cosma, Haris Krassos, Fotini Christofi, Maria Afxentiou, Rebecca Kokkinofita
  - 1Department of Forensic Chemistry and Toxicology, Cyprus State General Laboratory, 2Department of Forensic Chemistry and Toxicology, Cyprus State General Laboratory

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**HB4 09:08 Abstract ID 235**

**Title:** Large-scale study to evaluate the variability in elimination rate of the direct alcohol marker phosphatidylethanol during one month of abstinence.

**Authors:** Katleen Van Uytfanghe, UGent, FFW, Laboratory for Toxicology

- Katleen Van Uytfanghe, Liesl Heughebaert, Christophe Stove
  - UGent, FFW, Laboratory for Toxicology

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**HB5 09:20 Abstract ID 293**

**Title:** Prevalence of synthetic cannabinoid use among persons undergoing drug testing for cannabis – the impact of new NPS legislation in Germany.

**Authors:** Michaela J. Sommer, Institute of Forensic Medicine - Forensic Toxicology

- Michaela J. Sommer, Svenja Staudt, Florian Franz, Belal Haschimi, Bernd Schwarze, Volker Auwärter
  - 1Institute of Forensic Medicine - Forensic Toxicology, 2Forensic Toxicology, Institute of Forensic Medicine, University Erlangen-Nuremberg, Erlangen, Germany, 3Forensic Toxicology, Institute of Forensic Medicine, Medical Center - University of Freiburg, Freiburg, Germany

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**HB6 09:32 Abstract ID 305**

**Title:** Sales of CBD Containing Hemp Products, a New Trend in France.

**Authors:** Jérémy Lelong, chu poitiers

- Jérémy Lelong, Ysé Jagailloux, Bertrand Brunet
  - chu poitiers
HB7  09:44  **Abstract ID  400**  
Drug driving per se limits in England and Wales: A review of data from over 9,000 cases.  
Matthew Christopher  
Eurofins Forensic Services  
Matthew Christopher, Emily Evans, Susan Grosse, Elizabeth Spencer, Neville Isles  
Eurofins Forensic Services

HB8  09:56  **Abstract ID  448**  
THC and CBD concentrations in serum following single and repeated administration of "light cannabis".  
Filippo Pirani  
Section of Legal Medicine, Department of Excellence SBSP, Università Politecnica delle Marche, Ancona, Italy  
Filippo Pirani, Alfredo Fabrizio Lo Faro, Flaminia Pantano, Roberta Pacifici, Simona Pichini, Francesco Paolo Busardò  
1Section of Legal Medicine, Department of Excellence SBSP, Università Politecnica delle Marche, Ancona, Italy, 2Unit of Forensic Toxicology, Section of Legal Medicine, Department of Excellence SBSP, Università Politecnica delle Marche, Ancona, Italy, 3Unit of Forensic Toxicology Sapienza University of Rome, 4National Centre on Addiction and Doping, Istituto Superiore di Sanità, Rome, Italy

HB9  10:08  **Abstract ID  512**  
Predominance of illicit drugs and ethanol in blood among suspected drug-impaired drivers in Western Switzerland.  
Marc Augsburger  
University Center of Legal Medicine, Lausanne-Geneva  
Marc Augsburger, Nicolas Donzè, Frank Sporkert, Christèle Widmer, Julien Dégion, Aurélien Thomas  
1University Center of Legal Medicine, Lausanne-Geneva, 2Valais Hospital, Central Institute

10:20  BREAK

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**DRUG-FACILITATED CRIME**

**Oral Session I**

**THURSDAY 5th September 10:50 - 12:20 (Hall 1)**

**Moderators**  
Pascal Kintz (France) & David Osselton (UK)

**DFC1  10:56**  
**Abstract ID  151**  
Prevalence of drugs and alcohol in sexual assault cases and the interest of the toxicological analysis in the judicial investigation and final judgement.  
Sarah Wille  
NICC  
Sarah Wille, Karolien Van Dijck, Charlotte Aelbrecht, Vincent DiFazio, Maria Del Mar Ramirez-Fernandez, Roselien Crab, Lore George, Antje Van Assche, Vanessa Vanvooren, Nele Samyn  
NICC

**DFC2  11:08**  
**Abstract ID  238**  
The Unconventional side of Forensic Toxicology in the UK.  
Mark Tyler  
Eurofins Forensic Services  
Mark Tyler  
Eurofins Forensic Services
Abstract ID  414
Baseline Endogenous GHB Concentrations in Hair.
Mark Miller
FBI
Mark Miller1, Erin Strickland2, Jennifer Thomas3, Mike Smith1, Marc LeBeau1, Eugene Peters1
1FBI, 2FBI ORISE

Abstract ID  397
Tramadol distribution in hair after a single dose – a controlled study over four months.
Sys Johansen
Forensic Medicine, University of Copenhagen
Sys Johansen1, Linda Le Dang2, Marie Nielsen2, Pernilla Haage3, Frederik Kugelberg4, Robert Kronstrand4
1Forensic Medicine, University of Copenhagen, 2University of Copenhagen, 3National Board of Forensic Medicine, Linköping, Sweden, 4Department of Medical and Health Sciences, Linköping University, Linköping, Sweden

Abstract ID  398
Jessica Welter-Luedeke
Institute of Legal Medicine, Ludwig-Maximilians-University Munich
Jessica Welter-Luedeke1, Eva Koch2, Sebastian Halter3, Matthias Graw2, Liane D. Paul2
1Institute of Legal Medicine, Ludwig-Maximilians-University Munich, 2Institute of Legal Medicine, Forensic Toxicology, Ludwig-Maximilians-University Munich, 3Institute of Forensic Medicine, Forensic Toxicology, Medical Center - University of Freiburg, Germany

Abstract ID  82
Detection of Drug-Facilitated Sexual Assault (DFSA) compounds from adulterated beverages using monolithic silica adsorbents and multimode inlet GC-MS/MS.
Brian Waters
Fukuoka University
Brian Waters1, Yuki Sakamoto2, Kenji Hara1, Mio Takayama1, Aya Matsusue1, Masayuki Kashiwagi1, Shin-ichi Kubo1
1Fukuoka University, 2Shimadzu Corporation

Abstract ID  97
Research on screening methods of common hypnotic drugs and metabolites: application to forensic cases.
Leiping Zhang
Institute of Forensic Science, Ministry of Public Security, China
Leiping Zhang1, Jiqiang Ma2, Duoqi Xu3, Lijing Zhong2
1Institute of Forensic Science, Ministry of Public Security, China, 2Institute of Forensic Science, Ministry of Public Security, Beijing, China, 3China university of political science and law
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<th>Moderators</th>
<th>Wayne Jones (Sweden) &amp; Christophe Stove (Belgium)</th>
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<td><strong>HB10</strong> 13:20</td>
<td>Abstract ID 365</td>
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<td></td>
<td>The United Nations Office on Drugs and Crime Early Warning Advisory on New Psychoactive Substances.</td>
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<td>Conor Crean</td>
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<td>United Nations Office on Drugs and Crime</td>
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<td>United Nations Office on Drugs and Crime</td>
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<td><strong>HB11</strong> 13:32</td>
<td>Abstract ID 502</td>
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<tr>
<td></td>
<td>Application of Hair Analysis to Document Illegal 5-Methoxy-N,N-dissopropyltrptamine (5-MeO-DiPT) use.</td>
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<td>Ping Xiang</td>
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<td>Academy of Forensic Science, China</td>
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<td>Yan Shi, Ping Xiang, Roujia Wang, Min Shen</td>
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<td>Academy of Forensic Science, China</td>
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<td><strong>HB12</strong> 13:44</td>
<td>Abstract ID 492</td>
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<td>Large discrepancies of methamphetamine immunoassay screening and GC-MS confirmation results caused by presence of optically pure R-(-)-MA in serum samples.</td>
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<td>Lisa Oßowski</td>
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<td>University Hospital Jena, Department of Forensic Medicine</td>
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<td>Lisa Oßowski, Grit Kießling, Julia Dinger, Frank Theodor Peters</td>
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<td>University Hospital Jena, Department of Forensic Medicine</td>
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<td><strong>HB13</strong> 13:56</td>
<td>Cancelled</td>
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<td>Abstract ID 542</td>
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<td>The new trends of drug diversion and drug use in the Western Region of Algeria.</td>
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<td>Bendjamaa Atika</td>
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<td>Faculty of Medicine - University Oran 1</td>
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<td>Bendjamaa Atika, Arab Fatima Zohra, Rafa Fethia, Kherraf Leila, Khelifi Soumia, Rezk-kallah Haciba</td>
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<td></td>
<td>1Faculty of Medicine - University Oran 1, 2University Hospital of Oran</td>
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<td><strong>HB14</strong> 14:08</td>
<td>Abstract ID 93</td>
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<td>The rise of flubromazolam as counterfeit Xanax®: detecting and managing the emergence of an NPS.</td>
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<td>Marie-Jo Lajoie</td>
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<td>Marie-Jo Lajoie, Laurie Bedard, Brigitte Desharnais, Catherine Lavallée, Pascal Mireault</td>
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<td>1LSJML, 2Laboratoire de sciences judiciaires et de médecine légale</td>
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<td><strong>HB15</strong> 14:20</td>
<td>Abstract ID 355</td>
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<td>Drug testing in hair: a powerful tool to approach the epidemiology of polydrug use.</td>
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<td>Marie José Burgueño</td>
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<td>National Institute of Toxicology and Forensic Sciences, Madrid, Spain</td>
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<td>M José Burgueño, Sergio Sánchez, M Ángeles Castro, Ramona Mateos-Campos</td>
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<td>1National Institute of Toxicology and Forensic Sciences, Madrid, Spain, 2University of Salamanca, Faculty of Pharmacy, Pharmaceutical Chemistry, Salamanca, Spain, 3University of Salamanca, Faculty of Pharmacy, Preventive Medicine and Public Health, Salamanca, Spain</td>
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The emergence of deschloro-N-ethyl-ketamine, a ketamine analog, in drug seizures and drug driving cases in Hong Kong.

Wing Chi Cheng
Government Laboratory of Hong Kong

Wing Chi Cheng, Kwok Leung Dao
Government Laboratory of Hong Kong

The Presentation of Hair Strand Analysis Evidence in Court.

Alexander Robert Forrest
University of Sheffield

Alexander Robert Forrest\(^1\), Paul Hunter\(^2\)
\(^1\)University of Sheffield, \(^2\)Forensic testing Services Ltd.

Toxicological Findings from Alleged Sexual Assault Victims in Native American Communities from 2011 to 2018.

Marc LeBeau
FBI Laboratory

Marc LeBeau, Aleonna Scott, Madeline Montgomery, Cynthia Morris-Kukoski, Jason Schaff, Rena Hammer, Preston Lowe
FBI Laboratory


Helena M. Teixeira
Forensic Chemistry and Toxicology Service; National Institute of Legal Medicine and Forensic Sciences; Porto; Portugal.

Sónia Tarelho\(^1\), André Castro\(^1\), Helena M. Teixeira\(^2\), João Miguel Franco\(^3\)
\(^1\)Forensic Chemistry and Toxicology Service; National Institute of Legal Medicine and Forensic Sciences; Porto; Portugal., \(^2\)Department of Research, Training and Documentation of the National Institute of Legal Medicine and Forensic Sciences; Medicine Faculty of the University of Coimbra; Portugal., \(^3\)Forensic Chemistry and Toxicology Service; National Institute of Legal Medicine and Forensic Sciences; Lisbon; Portugal.

Pharmacological evaluation and detection of the recently emerged synthetic cannabinoid 4F-MDMB-BINACA in ‘legal-high’ products and human urine specimens.

Belal Haschimi
Forensic Toxicology, Institute of Forensic Medicine, Medical Center, University of Freiburg, Germany

Belal Haschimi\(^1\), Lukas Mogler\(^2\), Sebastian Halter\(^2\), Arianna Giorgetti\(^3\), Bernd Schwarze\(^4\), Folker Westphal\(^5\), Svenja Fischmann\(^5\), Volker Auaerter\(^2\)
\(^1\)Forensic Toxicology, Institute of Forensic Medicine, Medical Center, University of Freiburg, Germany, \(^2\)Forensic Toxicology, Institute of Forensic Medicine, Medical Center - University of Freiburg, Germany, \(^3\)Legal Medicine and Toxicology, University Hospital of Padova, Italy, \(^4\)Institute of Forensic Medicine, Erlangen, Germany, \(^5\)State Bureau of Criminal Investigation Schleswig-Holstein, Kiel, Germany)
Ten Years of Experience with Synthetic Cannabinoids in the United States.

Melissa Fogarty
CFSRE

Melissa Fogarty1, Alex Krotulski1, Sherri Kacinko2, Barry Logan3
1CFSRE, 2NMS Labs, 3CFSRE/NMS Labs

Wastewater-based epidemiology combined with forensic toxicological information: The approach exemplified by cocaine and methamphetamine use in Finland.

Teemu Gunnar
National Institute for Health and Welfare / Forensic Toxicology Unit

Teemu Gunnar, Aino Kankaanpää, Kimmo Kuoppasalmi
National Institute for Health and Welfare / Forensic Toxicology Unit

Screening of New Psychoactive Substances, THC and cocaine in urine samples obtained at two music festivals in the metropolitan area.

Eleuterio Umpiérrez
Facultad de Química

Eleuterio Umpiérrez1, Inês Petrini1, Daniela Diaz1, Lucia Dellepiane1, Magela Banchero1, Fernanda Iglesias2, Maria José Castro1
1Facultad de Química, 2Ex-Facultad de Química

A Voice in the Wilderness: Unexpected Drugs Found in Unregulated Cannabidiol E-cigarette Products by the Unsuspecting Public.

Michelle Peace
Virginia Commonwealth University

Michelle Peace, Justin Poklis, Haley Mulder, Shelle Butler, Samuel Miller
Virginia Commonwealth University

Fentanyl and Analogs: A Study of US Controlled Substances Act (CSA) Scheduling and Trends of Positive Results in Two Testing Populations.

Ruth Winecker
RTI International

Ruth Winecker1, Jeri Ropero-Miller1, Dustin Yeatman2, Hope Smiley-McDonald3, David Heller3, Justin Brower1
1RTI International, 2PBSO, 3NC-OCME

Cognitive bias in forensic toxicology.

Hilary Hamnett
University of Lincoln

Hilary Hamnett1, Rachael Jack2
1University of Lincoln, 2University of Glasgow

Fast UHPLC-MS/MS screening for 87 NPS and 32 classic illicit drugs in whole blood, serum, urine and hair.

Francesco Paolo Busardò
University Politecnica delle Marche

Simona Pichini1, Giulio Mancocci2, Roberta Tittarelli2, Massimo Gottardi3, Francesco Paolo Busardò3, Raffaele Giorgetti5
1National Institute of Health, 2Sapienza University and School of Law, Camerino University, 3Sapienza University, 4Comedical SRL, 5Università Politecnica delle Marche

CLOSE

17:20 - 17.40 UKIAFT Business Meeting
CONFIDENCE IN THE LAB

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## TOXICOLOGY IN SPORT

### Oral Session

**FRIDAY 6th September 08:30 - 10:20 (Hall 1)**

<table>
<thead>
<tr>
<th>Moderators</th>
<th>08:32</th>
<th>Abstract ID 302</th>
</tr>
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<tr>
<td>David Cowan (UK) &amp; Maria Parr (Germany)</td>
<td><strong>SP1</strong></td>
<td>Tracing back drug misuse – proper metabolite identification requires synthesis.</td>
</tr>
<tr>
<td>Maria Kristina Parr</td>
<td>Freie Universitaet Berlin</td>
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<tr>
<td>Maria Kristina Parr¹, Giuseppe La Piana¹, Anna Stoll¹, Jan Felix Joseph¹, Steffen Loke¹, Nils Schloerer², Xavier de la Torre³, Francesco Botrè⁴</td>
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<tr>
<td>¹Freie Universiathet Berlin, ²University of Cologne, ³Laboratorio Antidoping FMSI, Rome, ⁴Laboratorio Antidoping FMSI Rome and ‘Sapienza’ University of Rome, Department of Experimental Medicine</td>
<td></td>
<td></td>
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</tbody>
</table>

| 08:44 | Abstract ID 14 |
| **SP2** | Long-term abuse of clenbuterol and stanozolol: post mortem investigations. |
| Pascal Kintz | X-Pertise Consulting |
| Pascal Kintz¹, Alice Ameline², Laurie Gheddar², Véronique Dumestre³, Jean-Sébastien Raul² |
| ¹X-Pertise Consulting, ²Institut de médecine légale de Strasbourg, ³Laboratoire ToxGen, Bordeaux |

| 08:56 | Abstract ID 543 |
| **SP3** | Microsampling as a promising anti-doping strategy for the analysis of peptide hormones and growth factors. |
| Michele Protti | University of Bologna |
| Laura Mercolini¹, Michele Protti¹, Roberto Mandrioli¹, Paolo Sberna², James Rudge³ |
| ¹University of Bologna, ²University of Delft, ³Neoteryx |

| 09:08 | Abstract ID 392 |
| **SP4** | Performance-enhancing substances used by bodybuilders. |
| Jean Claude Alvarez | APHP and University of Versailles SQ |
| Jean Claude Alvarez¹, Nicolas Fabresse², Adeline Knapp³, Charlotte Mayer³, Paméla Dugues³, Islam Amine Larabi⁴ |
| ¹APHP and University of Versailles SQ, ²University Versailles, ³APHP, ⁴APHP and University Versailles |

| 09:20 | Abstract ID 300 |
| **SP5** | Discrimination of microbial produced boldenone from its exogenous administration – examples from equine doping control. |
| Marjaana Viljanto | LGC |
| Marjaana Viljanto¹, Andrew Kicman², Chris Walker², Kim Wolff², James Scarth¹ |
| ¹LGC, ²Drug Control Centre, King’s College London |

| 09:32 | Abstract ID 117 |
| **SP6** | No Pain No Gain – Influence of Pain Killers on the Anabolic Steroid Profile. |
| Anna Stoll | Freie Universitaet Berlin, Institute of Pharmacy |
| Anna Stoll¹, Michele Iannone², Xavier de la Torre², Francesco Botrè³, Maria Kristina Parr¹ |
| ¹Freie Universiathet Berlin, Institute of Pharmacy, ²Laboratorio Antidoping FMSI Rome, ³Laboratorio Antidoping FMSI Rome and Dipartimento di Medicina Sperimentale, “Sapienza” Università di Roma |
**SP7 09:44**

**Abstract ID 536**
Towards non-targeted LC-HRMS screening for improved drug surveillance.

**Bethany Keen**
University of Technology, Sydney

Bethany Keen¹, Shanlin Fu¹, Adam Cawley², James Pyke², Chris Fouracre³

¹University of Technology, Sydney, ²Racing NSW, ³Agilent Technologies

**SP8 09:56**

**Abstract ID 32**
Testing for an anabolic androgenic steroid, stanozolol, in hair collected from 5 different anatomical regions.

**Laurie Gheddar**
Institut de Médecine Légale

Laurie Gheddar¹, Alice Ameline¹, Jean-Sébastien Raul¹, Pascal Kintz²

¹Institut de Médecine Légale, ²X-Pertise

**SP9 10:08**

**Abstract ID 471**
Voluntary or accidental doping? That’s the problem.

**Donata Favretto**
University of Padova

Donata Favretto¹, Susanna Vogliardi², Giulia Stocchero³, Giuseppe Pieraccini⁴

¹University of Padova, ²University of Padova, ³Azienda Ospedaliera di Padova, ⁴University of Florence

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**NEW INNOVATIONS & NOVEL RESEARCH**

**Oral Session I**

**FRIDAY 6th September 10:50 - 12:20 (Hall 1)**

**Moderators**
Luca Morini (Italy) & Markus Meyer (Germany)

**NE1 10:56**

**Abstract ID 201**
A Point System for “Identifications” in Forensic Toxicology Laboratories.

**Marilyn Huestis**
Lambert Center for the Study of Medicinal Cannabis and Hemp, Thomas Jefferson University

Marilyn Huestis¹, Marc LeBeau¹, Marilyn Huestis²

¹FBI Laboratory, ²Lambert Center for the Study of Medicinal Cannabis and Hemp, Thomas Jefferson University

**NE2 11:08**

**Abstract ID 129**
Sensitive and selective screening of 143 fentanyl-related substances in biological samples using LC-Q-TOF-MS coupled with ion mobility separation.

**Ruri Kikura-Hanajiri**
National Institute of Health Sciences

Ruri Kikura-Hanajiri, Maiko Kawamura, Takashi Hakamatsuka

National Institute of Health Sciences

**NE3 11:20**

**Abstract ID 433**
Detection of Fentanyl and Methamphetamine using surface enhanced Raman spectroscopy.

**Rhiannon Alder**
University of Technology Sydney

Rhiannon Alder, Linda Xiao, Shanlin Fu

University of Technology Sydney
Abstract ID 303
Analyte quantification in Dried Blood Spots deposited on non-standardized materials with subsequent blood volume estimation.

Lina Gessner
Institute of Legal Medicine, Cologne

Lina Gessner1, Ursula Telgheder2, Hilke Andresen-Streichert1, Katja Mercer-Chalmers-Bender4, Martin Juebner3
1Institute of Legal Medicine, Cologne, 2University of Duisburg-Essen, Faculty of Chemistry, Instrumental Analytical Chemistry, Essen, Germany, 3Institute of Legal Medicine, Medical Faculty, University of Cologne, Cologne, Germany, 4Institute of Legal Medicine of the University of Basel, Forensic Chemistry and Toxicology, Basel, Switzerland

Abstract ID 324
ToF-SIMS and MALDI Analysis of Longitudinal - and Cross Sections of Single Hairs as a Tool for Gaining Insight into the Contamination Issue.

Robert Erne
Zurich Institute of Forensic Medicine (ZIFM)

Robert Erne1, Laetitia Bernard2, Markus R. Baumgartner3, Thomas Kraemer3
1Zurich Institute of Forensic Medicine (ZIFM), 2Empa, Duebendorf, Switzerland, 3Zurich Institute of Forensic Medicine (ZIFM), Switzerland

Abstract ID 357
What we thought we knew – Master class on MSMS-spectra interpretation of fentanyl metabolites.

Svante Vikingsson
National Board of Forensic Medicine / Linköping University

Svante Vikingsson1, Jakob Wallgren2, Anna Åstrand2, Shimpei Watanabe3, Robert Kronstrand1, Henrik Gréen1
1National Board of Forensic Medicine / Linköping University, 2Linköping University, 3National Board of Forensic Medicine

Abstract ID 391
A Novel Serotonin Receptor Bio-Assay For The Activity-Profiling And Detection Of Hallucinogenic NPS?

Eline Pottie
Ghent University - Laboratory of Toxicology

Eline Pottie, Katleen Van Uytfanghe, Annelies Cannaert, Christophe Stove
Ghent University - Laboratory of Toxicology
EMPLOYMENT & OCCUPATIONAL
Oral Session
FRIDAY 6th September 13:20 - 14:50 (Hall 1)

Moderators
Sumandeep Rana (USA) & Peter Akrill (UK)

EO1  13:20
CANCELLLED

Abstract ID 164
Cocaine and related compounds in the hair of coca leaf chewers, coca tea drinkers and their
comparison with cocaine addicts by RIA, UPLC-DAD-MS/MS.

Luis Ferrari
UM University

Luis Ferrari¹, Pablo Escudero², Leda Giannuzzi³
¹UM University, ²LAM CENTER, ³National University of La Plata

EO2  13:32

Abstract ID 552
Detection of delta-8-THC-COOH in urine samples and its implications in workplace drug testing.

Sumandeep Rana
Redwood Toxicology Laboratory

Garry Brent Dawson, Benadetta Njau, Sumandeep Rana
Redwood Toxicology Laboratory

EO3  13:44

Abstract ID 203
Workplace drug testing in Australia – dealing with prescription and non-prescription
medications, dilute urine specimens and attempted adulteration.

Sam Bruschi
AusHealth

John Edwards, Sam Bruschi
AusHealth

EO4  13:56

Abstract ID 330
Prevention of False Negativity with Creatinine Normalization.

Melike Aydoğdu
Ege University

Melike Aydoğdu¹, Sema Oral², Serap Annette Akgür³
¹Ege University, ²Health Sciences University Bursa Higher Specialization Training and Research
Hospital, Bursa, Turkey, ³Ege University, Institute on Drug Abuse, Toxicology and Pharmaceutical
Science, Izmir, Turkey

EO5  14:08

Abstract ID 326
Deep Learning as a tool for classification of biological samples measured by HRMS: Detection of
adulterated urine samples and cosmetically treated hair samples.

Gabriel Streun
Forensic Pharmacology & Toxicology, Institute of Forensic Medicine, University of Zurich

Gabriel Streun, Marco Elmiger, Lisa Eisenbeiss, Andrea Steuer, Thomas Krämer
Forensic Pharmacology & Toxicology, Institute of Forensic Medicine, University of Zurich

EO6  14:20

Abstract ID 403
Recreational vs. medical use of amphetamine-type phenethylamines: Enantioselective
analysis of metabolites in urine samples.

Hannes Max Schwelm
Forensic Toxicology, Institute of Forensic Medicine, Medical Center – University of Freiburg, Germany

Hannes Max Schwelm, Christina Grumann, Volker Auwärter, Merja A. Neukamm
Forensic Toxicology, Institute of Forensic Medicine, Medical Center – University of Freiburg, Germany
Abstract ID  5
Fully automated determination of phosphatidylethanol 16:0/18:1 and 16:0/18:2 in dried blood spots.
Marc Luginbühl
Institute of Forensic Medicine, Bern
Marc Luginbühl1, Stefan Gaugler2, Wolfgang Weinmann1
1Institute of Forensic Medicine, Bern, 2CAMAG

Abstract ID  298
Incidence of drugs of abuse within the Australian Workforce subject to random drug testing.
Andrew Leibie
Self
Andrew Leibie
Self

NEW INNOVATIONS & NOVEL RESEARCH
Oral Session II
Friday 6th September 15:20 - 16:20 (Hall 1)

Moderators
Susan Grosse (UK) & Brigitte Desharnais (Canada)

Abstract ID  30
Handling uncertainty of measurement in qualitative methods: a tool to help get ready for ISO 17025:2017 certification.
Brigitte Desharnais
Concordia University
Brigitte Desharnais1, Félix Camirand Lemyre2, Pascal Mireault3, Cameron D. Skinner1
1Concordia University, 2Université de Sherbrooke, 3Laboratoire de sciences judiciaires et de médecine légale

Abstract ID  65
Multidetermination of drugs of abuse in human hair using restricted access supramolecular solvent combined with LC-MS/MS.
Gedifew Nigatu Beza
St. Paul’s Hospital Millennium Medical College
Gedifew Nigatu Beza1, Soledad Rubio2
1St. Paul’s Hospital Millennium Medical College, 2University of Cordoba, Spain

Abstract ID  396
P4 strategic production of NPS reference materials – Eurostars PSYCHOMICS Project.
Huiling Liu
Chiron AS
Huiling Liu, Matthew Connolly Connolly, Alexey Gorovoy, Olga Gozhina, Jenny Button, Jon Eigill Johansen
Chiron AS
Abstract ID 440
Enantiospecific synthesis, separation, detection and pharmacological assessment of carboxamide-type synthetic cannabinoid receptor agonists in seized samples.

Lysbeth Antonides
University of Dundee

Craig Mckenzie\(^1\), Lysbeth Antonides\(^2\), Annelies Cannaert\(^3\), Caitlyn Norman\(^4\), Loelia Vives\(^5\), Aidan Harrison\(^6\), Andrew Costello\(^7\), Niamh Nic Daeid\(^8\), Christophe Stove\(^9\), Oliver Sutcliffe\(^10\)

\(^1\)University of Dundee, \(^2\)Leverhulme Research Centre for Forensic Science, University of Dundee, \(^3\)Laborotory of Toxicology, Ghent University, \(^4\)Forensic Drug Research Group, Centre of Anatomy and Human Identification, University of Dundee, \(^5\)Forensic Drug Research Group, Centre of Anatomy and Human Identification, University of Dundee, \(^6\)Phenomenex, \(^7\)Greater Manchester Police, \(^8\)Laboratory of Toxicology, Faculty of Pharmaceutical Sciences, Ghent University, \(^9\)Manchester Metropolitan University

Abstract ID 222
Searching matches for time-of-flight spectra in an Orbitrap spectral database: results from a study simulating tentative identification of unknowns.

Anna Pelander
National Institute for Health and Welfare

Anna Pelander\(^1\), Pirkko Kriikku\(^1\), Sara Pasanen\(^2\)

\(^1\)National Institute for Health and Welfare, \(^2\)University of Helsinki
ABOUT THE JOURNAL

The *Journal of Analytical Toxicology* is an international toxicology journal devoted to the timely dissemination of scientific communications concerning potentially toxic substances and drug identification, isolation, and quantitation. Since its inception in 1977, the *Journal of Analytical Toxicology* has striven to present state-of-the-art techniques used in toxicology labs.

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The TIAFT 2019 Special Issue and manuscript submission deadline is December 16th. Submit to bit.ly/SubmitTIAFT
TUESDAY 3rd September 2019

Hall 8a.

Time: 5:45-7:15pm.*

Analytical strategies to identify analytes of forensic interest in routine pharmacotoxicology laboratories.
Speaker: Dr. Simona Pichini, Analytical Pharmacotoxicology Unit - National Centre on Addiction and Doping, National Institute of Health, Rome Italy.
Target Quantitation and Non-Target Forensic Toxicology Screening using High Resolution Mass Spectrometry on the Agilent6546 Q-TOF.
Speaker: Gordon Ross, Agilent Technologies, Cheadle UK.

Hall 7.

Time: 5:45-7:15pm.*

“Efficient workflow in forensic toxicology supported by modern software solution”.
Speaker: Dr. rer. nat. Jochen Beyer - Department Forensic Toxicology from the University of St. Gallen.
Speaker: Dr. Michael Busch senior project manager Dorner Health Solutions

Hall 6.

Time: 5:45-7:15pm.*

“Drugs of Abuse Testing by LC-MS/MS”.
Investigation of an LC-MS/MS Drugs of Abuse Assay by High Resolution LC-MS.
Speaker: Dr Lewis Couchman, Analytical Services International Ltd, UK.
Speaker: Dr Christoph Geffert, Labor Staber Dresden-Klipphausen, Germany.

WEDNESDAY 4th September 2019

Hall 8a.

Time: 8:30-10:00am*

“Confident Results in Toxicology. Innovative solutions for fast and sensitive drugs of abuse screening”.
Analysis on the drivers involved in road incidents and the analytical approach for determining of DAT abuse.
Speaker: Dr. Guido Pelletti MD, University of Bologna, Bologna (UNIBO), Italy.
Modernizing GC sampling solutions to serve productivity and result defensibility in forensic laboratories.
Speaker: Daniela Cavagnino, Thermo Fisher Scientific, Rodano (MI), Italy.

*Note: Main session times shown, please see vendors for any additional networking timings
**Vendor Seminars**

**WEDNESDAY 4th September 2019**

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**Hall 7.**

**Time: 8:30-10:00am**

Current analytical methods for the detection of new psychoactive substances (NPS).
Speaker: Prof. Dr. Volker Auwärter, University Medical Center Freiburg, Institute of Forensic Medicine.
Testing for fentanyl - When the targeted analysis is not enough.
Speaker: Alberto Salomone, Associate Professor at the University of Turin, Department of Chemistry.

---

**Hall 8a.**

**Time: 10:20-11:50am**

“Toxicology Screening for postmortem samples and identifying the unexpected”.

**UHPLC-MSn in Forensic Toxicology: screening of toxicologically relevant substances in biological samples at Bureau of Forensic Medicine of Moscow region.**
Speaker: Dr. Natalia Krupina, Head of Department of Forensic Chemistry, Bureau of Forensic Medicine of Moscow region.

**The unknown known or the known unknown?**
Speaker: Laura Huppertz, Institute of Forensic Medicine, Medical Center - University of Freiburg.

**Rapid Detection and Separation Isomeric of Fentanyl Analogs using GC APCI timsTOF MS.**
Speaker: Elisa Shoff, Miami-Dade Medical Examiner Department & Florida International University, Miami.

---

**Hall 6.**

**Time: 10:20-11:50am**

“The science behind forensics; how technology is making a meaningful difference!”

**How can we make workflows in toxicology and forensics screening better?**
Speaker: Franck Saint-Marcoux / CHU de Limoges / Biological and Medico-Legal Toxicology Officer / Department of Pharmacology, Toxicology and Pharmacovigilance.

**Fully automated alcohol abuse and illicit drug testing from dried blood spots.**
Speaker: Stefan Gaugler / CAMAG / Product Manager DBS.

**A new QToF for easier routine toxicology and forensics analysis**
Speaker: Mikael Levi / Shimadzu Corp. / MS Business Unit

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*Note: Main session times shown, please see vendors for any additional networking timings*
THURSDAY 5th September 2019

Hall 8a.

Time: 5:45-7:15pm.*

Analytical evidence to show letters impregnated with NPS are a means of getting drugs to inmates within the UK prison service.
Speaker: Dr Loretta Ford, Consultant Clinical Scientist, Department of Clinical Biochemistry, City Hospital Birmingham, UK.

Current trends in NPS.
Speaker: Dr Craig McKenzie, Senior Lecturer in Forensic Chemistry, Forensic Drug Research Group, Centre for Anatomy and Human Identification, School of Science and Engineering, University of Dundee, Dundee, Scotland.

---

Hall 6.

Time: 5:45-7:15pm.*

Automation seminar - “Positive pressure technology for fast and simple sample prep”.
Speaker: Karsten Liegmann, Senior Application Chemist, Tecan SpeWare, USA

Topics:
Broad spectrum SPE in multiple matrices using positive pressure technology.
The potential utility of differential elution to reduce ion suppression from samples with high gabapentin concentrations.

New extraction strategies and column chemistry to cover challenging analytes:
• Ethyl Glucuronide/Ethyl Sulfate (EtG/EtS)
• Thyroid Hormones (T3, rT3 and T4)
• Catecholamines

Other application areas:
• Proteomics
• Protein purification
• Metabolite screening

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Hall 7.

Time: 5:45-7:15pm.*

“Fast and Accurate Analysis of Controlled and non-Controlled Substances by Solid Deposition GC-FTIR.”

Solid-Phase GC-FTIR for Differentiation of Closely Related NPS Isomers.
Speaker: Jeffrey Kearney, Spectra Analysis Instruments, Inc.

Solid-phase deposition GC-FTIR for Reliable Identification of NPS on Seized Drugs and Human Urine Samples.
Speaker: Dr. Tania Salerno, BeSep S.r.l. c/o Department of Chemistry, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy.

*Note: Main session times shown, please see vendors for any additional networking timings
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Abstract ID 6
Increment in concentrations of Ethanol in Blood and Vitreous Humor found in the last three years in Argentina.
Karina Casares
Clara Inés Pereira, Karina Casares, Karina Lagrange, Lucrecia Orlando
Morgue Judicial de la Nación - Argentina

Abstract ID 17
Death of a newborn in a nursery: is hydroxyzine involved?
Frederic Aknouche
Frederic Aknouche, Frederic Aknouche
Laboratoire BARLA

Abstract ID 25
Total mercury determination by TDA AAS in body fluids and tissues for forensic purposes.
Teresa Lech
Teresa Lech1, Wioletta Turek2
1Institute of Forensic Research, 2Jagiellonian University, Department of Chemistry

Abstract ID 27
Quantification of U-47700 and its metabolites: N-desmethyl-U-47700 and N,N-didesmethyl-U-47700 in 12 autopsy blood samples employing SPE/LC-ESI-MS-MS.
Sebastian Rojek
Sebastian Rojek, Agnieszka Romanczuk, Karol Kula, Kamil Synowiec, Małgorzata Klys
Jagiellonian University Medical College, Department of Forensic Medicine

Abstract ID 31
Screening for synthetic cannabinoids should not be limited to blood samples - considerations supported by case of poisoning with AMB-FUBINACA and EMB-FUBINACA.
Piotr Adamowicz
Piotr Adamowicz1, Ewa Meissner2
1Institute of Forensic Research, 2Department of Forensic Medicine, Medical University of Lodz

Abstract ID 48
Old drugs in modern times: Opium poisoning fatalities in Western countries.
María Antonia Martínez
María Antonia Martínez, Salomé Ballesteros
National Institute of Toxicology and Forensic Sciences. Justice Ministry

Abstract ID 50
Identification and quantification of NPS: U-47700, N-ethylhexedrone, adinazolam, 4-CIC and 4-CMC in evidence and biological material using LC-MS/MS-case report.
Karolina Nowak
Karolina Nowak1, Marcin Zawadzki2, Paweł Szpot2, Tomasz Jurek1
1Department of Forensic Medicine, Faculty of Medicine, Wroclaw Medical University, Wroclaw, Poland; 2Department of Forensic Medicine, Faculty of Medicine, Wroclaw Medical University, Wroclaw, Poland

Abstract ID 52
Determination of boldenone in postmortem specimens including blood and urine samples using LC-MS/MS.
Dongeun Park
Meejung Park1, Juhyun Sim1, Eunmi Kim1, Dongeun Park2
1National Forensic Service, 2Daejeon Institute, National Forensic Service

Abstract ID 78
Deaths linked to synthetic cannabinoid - CUMYL-PEGACLONE.
Melynda Hargreaves
Melynda Hargreaves1, Matthew DiRago1, Kerry Crump1, Maria Pricone1, Samantha Joubert1, Linda Glowacki2, Matthew Lynch2, Noel Woodford2
1Victorian Institute of Forensic Medicine, 2Victorian Institute of Forensic Medicine, Department of Forensic Medicine
Abstract ID  81
Determination of 7 psychotropic drugs and metabolites in hair by LC-MS/MS: identification of quetiapine acute poisoning using hair root.
Hui Yan
Hui Yan, Ping Xiang, Jiaojiao Ji, Min Shen
Academy of Forensic Science, Shanghai Key laboratory of Forensic Medicine, China

Abstract ID  85
Stability studies of cocaine compounds in biological fluids during post-analysis custody.
Carmen Jurado
Carmen Jurado, Teresa Huertas, Teresa Soriano, Manuel Salguero
National Institute of Toxicology and Forensic Sciences

Abstract ID  91
Insufflation of Ethyl chloride: a case report.
Jennifer P Pascali
Jennifer P Pascali1, Diego Palumbo1, Paolo Fais2, Fabio Vaiano1, Elisabetta Bertol1
1University of Florence, 2University of Bologna

Abstract ID  120
Determination of drugs in histological material by liquid chromatography–tandem mass spectrometry.
Katarina Henning
Katarina Henning, Marek Dziadosz, Michael Klintschar, Jörg Teske
Hannover Medical School, Institute for Forensic Medicine

Abstract ID  127
Method for the quantification of heroin biomarkers and their metabolites using LC-MS-MS in postmortem specimens.
Ahmed Al-Asmari
Ahmed Al-Asmari
Ministry of Health, Saudi Arabia

Abstract ID  135
Integrating toxicology into the diagnosis of sudden cardiac death.
Bernardino Barcelo
Bernardino Barcelo1, Tomas Ripoll2, Ana Belén Garcia3, Damián Heine4, Isabel Gomila4, Estela García5
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Abstract ID  146
Kirsten Wiese Simonsen
Kirsten Wiese Simonsen, Brian Schou Rasmussen, Kristian Linnet
Section of Forensic Chemistry, Department of Forensic Medicine, Faculty of Health Sciences, University of Copenhagen

Abstract ID  155
A Death Involving Barbital – An Old Psychoactive Substance (OPS).
Daniel Isenschmid
Daniel Isenschmid1, Ayako Chan-Hosokawa2, Kristin Escobar Alvarenga3
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Abstract ID  182
Simultaneous determination of fentanyl, its metabolite norfentanyl, acetylfentanyl, butyrfentanyl, furanyllevetanyl and ofcentanyl in whole blood with GC-MS.
Sotiris Athanaseslis
Ioannis Papoutsis1, Nektaria Misailidi2, Panagiota Nikolaou3, Chaido Spiliopoulou2, Sotiris Athanaseslis2
1Department of Forensic medicine and Toxicology. National and Kapodistrian University of Athens, 2National and Kapodistrian University of Athens
Abstract ID 188
MDMA Intoxication in Potential donor with cardiac arrest – A case report.
Helena M. Teixeira
André Castro1, Sónia Tarelho2, Dina Almeida2, Lara Sousa2, João Miguel Franco3, Helena M. Teixeira4
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Abstract ID 204
New Psychoactive Substances (NPS)-Related Deaths in Taiwan During the 2012–2018 Period.
Yun-Chen Tsao
Yun-Chen Tsao1, Dong-Liang Lin1, Hsiu-Chuan Liu1, Ray H. Liu2
1Department of Forensic Toxicology, Institute of Forensic Medicine, Ministry of Justice, Taipei, Taiwan, 2Department of Criminal Justice, University of Alabama at Birmingham, Birmingham, AL, USA

Abstract ID 206
Xinxin Ren
Xinxin Ren, Aihua Wang, Linpei Dong, Zhongshan Yu, Ge Song, Xiaoping Hou
Institute of forensic science, ministry of public security, the People’s Republic of China

Abstract ID 207
Determination of the dimethyl sulfide in the hydrogen sulfide poisoned blood by headspace gas chromatograph mass spectrometry analysis(HS-GC/MS).
Guanfeng Cui
Guanfeng Cui, Fanglin Wang, Ying Dong
Institute of forensic science, ministry of public society, the People’s Republic of China

Abstract ID 210
Determination of Bromadiolone, Brodifacoum and Emamectin Benzoate in Human Whole Blood by LC-MS/MS.
Ying Dong
Ying Dong1, Fanglin Wang2, Guanfeng Cui2
1Institute of Forensic Science. Ministry of Public Security, 2Institute of forensic science, ministry of public society, the People’s Republic of China

Abstract ID 213
Suicides by inhalation of inert gasses: toxicological issues in 5 cases.
Alfredo Fabrizio Lo Faro
Alfredo Fabrizio Lo Faro, Filippo Pirani, Adriano Tagliabracci, Francesco Paolo Busardò, Raffaele Giorgetti
University *Politecnica delle Marche*

Abstract ID 218
Fatal intoxication by intravenously administrated extract of Ricinus communis seeds.
Marie Stankova
Marie Stankova, Petr Handlos, Martin Svidrnoch
University Hospital Ostrava

Abstract ID 221
Distribution of antidepressant drugs in vitreous humor.
Ioannis Papoutsis
Ioannis Papoutsis, Sotiris Athanaselis, Artemisia Dona, Panagiota Nikolaou, Chaido Spiliopoulou, Ioannis Papoutsis
Department of Forensic Medicine and Toxicology, Medical School, University of Athens

Abstract ID 224
Profile of poisoning cases in JSS Medical college & Hospital - A two year prospective study.
Chandrakanth Hungund
Chandrakanth Hungund, Chandrakanth Hungund Hungund, Arun Mohanram, Manjunatha Basappa
Jss Medical college JSS Academy of Higher education & Research

Abstract ID 225
Ethanol production by S. aureus under controlled experimental conditions: evaluation of applicability in postmortem cases.
Vassiliki Boumba
Vassiliki Boumba1, Glykeria Valivasi2, Hercules Sakkas1, Nikolaos Kourkoumelis1
1University of Ioannina, 2University of Ioannina
Abstract ID 227
Identification and quantification of antipsychotic in blood samples by LC-MS/MS: cases reports and data of 3 years of routine analysis.
Paula Proença
Paula Proença1, Carla Monteiro1, Carla Mustra1, Alda Claro1, João Miguel Franco1, Francisco Corte-Real2
1National Institute of Legal Medicine and Forensic Sciences, 2National Institute of Legal Medicine and Forensic Sciences; Faculty of Medicine, University of Coimbra

Abstract ID 228
Wonkyung Yang
Wonkyung Yang, Hyeyoung Choi, Moonhee Jang, Ji Hyun Kim, Ilichung Shin, Yuran Park, Seo Jin Kang
National Forensic Service

Abstract ID 230
Carbon Monoxide Poisoning: comparison of spectrophotometric and gas chromatographic methods for quantification under controlled storage conditions.
Stefania Oliverio
Stefania Oliverio1, Ariana Zeka2, Giovanni Leonardi3, Vincent Varlet1
1University Center of Legal Medicine Lausanne-Geneva, 2Brunel University London, 3London School of Hygiene and Tropical Medicine

Abstract ID 234
Identification of unique markers of 4-methylmethcathinone (4-MMC) degradation in putrefied biological matrices.
Shanlin Fu
Shanlin Fu1, Melissa Trujillo Uruena1, Rebekah Harrison1, Morgan Philip1, Zhiwen Wei2, Keming Yun2
1University of Technology Sydney, 2Shanxi Medical University, China

Abstract ID 237
Can UV-Vis spectroscopy be used to determine the % carboxyhaemoglobin in degraded blood and tissue samples?
Aaron McMillan
Aaron McMillan1, Lucy Sandford2, Abbie Oliver2, Dillon Brewster2, Fiona Perry3
1Eurofins Forensic Services, 2Eurofins Forensics Services

Abstract ID 240
Have point-of-care devices a role in forensic toxicology?
Duncan Stephen
Duncan Stephen, Duncan Stephen, Sille Lehepuu
NHS Grampian

Abstract ID 248
Suspected intoxication with nitrite: quantification of nitrite and nitrate in post-mortem human matrices.
Lauriane Drouin
Lauriane Drouin, Bart Ruiter, Caroliene Boone, Rogier Van der Hulst, Lennaert Borra, Ingrid Bosman
Netherlands Forensic Institute

Abstract ID 249
A case of death after the consumption of N-Ethylpentylone and MPHP.
Cláudia Margalho
Cláudia Margalho1, Alice Castanheira2, Fernando Castanheira2, Elisa Ferreira2, João Franco2, Francisco Corte-Real2
1National Institute of Legal Medicine and Forensic Sciences, IP; 2National Institute of Legal Medicine and Forensic Sciences

Abstract ID 252
Be aware of a barbiturate intoxication.
Corine Bethlehem
Corine Bethlehem, Isolede Vleut, Anne Van Rongen, Birgit Koch
Erasmus Medical Center

Abstract ID 256
Homicidal Poisoning Disguised as a Motor Vehicle Accident.
Amanda Jenkins
Amanda Jenkins1, Douglas Rohde2
1UMass Memorial Medical Center, 2Lake County Crime Laboratory

Abstract ID 273
Potential effects of the co-administration of AMB-FUBINACA and pFPP in New Zealand.
Diana Kappatos
Samantha Coward, Diana Kappatos
ESR
### Abstract ID 267
The first cases of death involving the novel synthetic cannabinoid 5F-Cumyl-PEGACLONE.

Arianna Giorgetti1, Lukas Mogler2, Vanessa Thoma2, Andreas Alt3, Daniel Rentsch4, Susanne Vogt2, Volker Auwärter2
1Legal Medicine and Toxicology, University-Hospital of Padova, Padova, Italy, 2Institute of Forensic Medicine, Forensic Toxicology, Medical Center - University of Freiburg, Germany, 3Institute of Legal Medicine, Ulm University, Ulm, Germany, 4Institute of Forensic Medicine, Forensic Toxicology, Medical Center - University of Rostock, St.-Georg-Str. 108, 18055, Rostock, Germany

### Abstract ID 292
Development and validation of an analytical method for volatiles with endogenous production in putrefaction situations.

Carla Monteiro
Mélanie Pinto1, Francisco Corte-Real2, Ermelinda Eusébio3, Carla Monteiro4
1Chemistry Department of the Faculty of Sciences and Technologies - University of Coimbra, 2Faculty of Medicine - University of Coimbra, 3Faculty of Sciences and Technologies - University of Coimbra, 4Forensic Chemistry and Toxicology Service; National Institute of Legal Medicine and Forensic Sciences; Portugal.

### Abstract ID 296
4-Methylethcathinone Related Death of a Slammer Documented with Postmortem Concentrations.

Jenny Becam
Jenny Becam1, Camille Richeval2, Jean François Wiart2, Jean Michel Gaulier2, Lucile Tuchtan3, Jacques Desfeux3, Anne Laure Pelissier1
1Service de médecine légale, CHU, Marseille, France, 2Unité Fonctionnelle de Toxicologie, CHU, Lille, 3Service de Médecine Légale, CHU, Marseille, France

### Abstract ID 304
Evaluation of CO-Hb quantification in post-mortem whole blood by oximetry and headspace gas chromatography with flame ionization detection.

Lena Kristoffersen
Lena Kristoffersen, Per Kristian Monge, Merete Vevelstad, Inger-Ann Hansen, Dag Helge Strand
Department of Forensic Sciences, Oslo University Hospital

### Abstract ID 308
The flood of “yellow heroin” (high-potency heroin) and the increase of heroin-related overdose deaths in north-east Italy during 2017-2018.

Giampietro Frison
Giampietro Frison, Luca Zamengo, Samuela Frasson, Chiara Bettin, Gianpaola Tedeschi, Flavio Zancanaro
Laboratory of Environmental Hygiene and Forensic Toxicology, DMPO Department, AULSS 3

### Abstract ID 318
Identification of acetyl-, acryl-, cyclopropyl-, isobutyryl- & 4F-isobutyrylfentanyl metabolites using in-house synthesized standards, hepatocytes & LC-QTOF-MS.

Henrik Green
Henrik Green1, Jakob Wallgren2, Johan Dahlén2, Anna Åstrand3, Robert Kronstrand4, Svante Vikingsson4
1National Board of Forensic Medicine, Sweden, 2Department of Physics, Chemistry and Biology, Linköping University, Linköping, Sweden, 3Division of Drug Research, Department of Medical and Health Sciences, Linköping University, Linköping, Sweden, 4Department of Forensic Genetics and Forensic Toxicology; National Board of Forensic Medicine, Linköping, Sweden

### Abstract ID 322
Case report of an atypical lethal overdose of naltrexone and its main metabolite 6-β-naltrexol in prison.

Cédric Priez Barallon.
Cédric Priez Barallon1, Vincent Lopez2, Cécile Moreau1, Baptiste Boyer2, Yvan Gaillard1
1LAT LUMTOX, 2Institute of Forensic Medicine, Clermont-Ferrand

### Abstract ID 327
Starvation suspicion in an enfant explored by postmortem biochemistry and testing for trace elements in hair.

Anne-Laure Alicot
Anne-Laure Alicot1, Cristian Palmieri2, Marc Augsburger2, Pascal Kintz2, Marie-Dominique Piercucci4, Valérie Baillif-Couniou1, Caroline Sastre1, Lucile Tuchtan4, Georges Leonetti1
1Faculté de Médecine, 2CURML, University Center of Legal Medicine, Lausanne, Switzerland, 3Institute of Legal Medicine, Strasbourg, France, 4Service de Médecine Légale, Faculté de Médecine, Marseille, France

### Abstract ID 328
Starvation suspicion in an enfant explored by postmortem biochemistry and testing for trace elements in hair.

Anne-Laure Alicot
Anne-Laure Alicot1, Cristian Palmieri2, Marc Augsburger2, Pascal Kintz2, Marie-Dominique Piercucci4, Valérie Baillif-Couniou4
1Faculté de Médecine, 2Unité de Toxicologie et Chimie Forensiques, CURML, Lausanne-Genève, Suisse, 3Institut Médicolégal, Strasbourg, France, 4Faculté de Médecine, Marseille, France
Abstract ID 340
Cases of poisoning or death related to suspicion of psychoactive substances or drugs abuse being analyzed at the Forensic Genetics Institute in 2018 by LC-MS/MS.
Jakub Czarny
Jakub Czarny1, Jadwiga Musial2, Natalia Galant3, Michal Raczkowski3, Renata Herman3, Magdalena Chrostowska3, Jolanta Powierska-Czarny2, Paulina Jerszy ska1, Barbara Przyjazna3
1Instytut Genetyki Sądowej Sp. z o.o., 2Institute of Forensic Genetics Jolanta Powierska-Czarny, 3Institute of Forensic Genetics I.I.c.

Abstract ID 360
A fatal case probably after an intramuscular injection of paliperidone.
Guillaume Drevin
Guillaume Drevin1, Bruno Ripault2, Jean-Baptiste Ballot-Ragaru2, Marie Deguigne3, Nathalie Jousset2, Benedicte Lelièvre3
1CHU Angers Department of pharmacology-Toxicology, 2CHU Angers Department of forensic medicine, 3CHU Angers Poison control center

Abstract ID 362
The prevalence of alcohol and other drugs in fatal road crashes in Victoria, Australia.
Dimitri Gerostamoulos
Dimitri Gerostamoulos1, Monica Perkins3, Paul Dietze3, Dhanya Nambiar3, Peter Cameron3, Dimitri Gerostamoulos1, Ben Beck2
1Victorian Institute of Forensic Medicine, 3Monash University, 4Burnet Institute

Abstract ID 363
When cultural differences breed a separate toxicological ecosystem: the province of Québec (Canada) and the opioids crisis.
Béatrice Garneau
Béatrice Garneau1, Jennifer Huynh2, Loralynn Martel2, Brigitte Desharnais1, Pascal Mireault1, André Lajeunesse2
1Laboratoire de sciences judiciaires et de médecine légale, 2Université du Québec à Trois-Rivières

Abstract ID 368
Research on the generation of Ethanol non-oxidation metabolites in various human blood samples.
Mingjun Yu
Mingjun Yu, Jiahui Chen, Xiuxiu Yang, Lingxiao Wang, Keming Yun, Zhiwen Wei
Shanxi Medical University

Abstract ID 371
Detection of poisonous phosphine gas in exhumed specimen.
Sohail Shahzad
Sohail Shahzad, Muhammad Imran, Sardar Ali Wattoo, Sana Hameed, Mohammad Sarwar, Mohammad Ashraf Tahir
PFS&A

Abstract ID 372
A dothiepin related death with unusually high tissue concentration.
Sohail Shahzad
Sohail Shahzad, Muhammad Imran, Sardar Ali Wattoo, Habib Anwar, Mohammad Sarwar, Mohammad Ashraf Tahir
PFS&A

Abstract ID 378
Streamlining Unknown Screening for Postmortem Analysis.
Aymeric Morla
Aymeric Morla1, Oscar Cabrices1, Adrian Taylor1, Dean Fritch2, Melanie Stauffer2, Derrick Schollenberger2
1SCIEX, 2Health Network Laboratories

Abstract ID 382
Orthodoxia Mastrogiani
Evdokia Brousató, Dimitrios-Pheidon Kevrekidis, Orthodoxia Mastrogiani, Amvrosios Orfanidis, Eleni Zagelidu, Nikolaos Raikos
Lab of forensic medicine and Toxicology, Medical School, Aristotle University of Thessaloniki

Abstract ID 383
Development of GC/MS method for THC determination in blood and application in real samples from dead drivers in Greece.
Orthodoxia Mastrogiani
Nikolaos Raikos1, Orthodoxia Mastrogiani1, Georgios Gretsos2, Amvrosios Orfanidis1, Evdokia Brousa2, Efstatia Antoniou2, Anca Mitrou1, Adamantios Krokos2
1Lab of Forensic Medicine and Toxicology, Medical School, Aristotle University of Thessaloniki, 2Lab of forensic Medicine and Toxicology, Medical School, Aristotle University of Thessaloniki

Abstract ID 394
Death by drowning after consumption of high dose of methadone: A case report.
Cláudia Margalho
Cláudia Margalho1, Elisa Ferreira2, Alice Castanheira2, João Franco2, Francisco Corte-Real2
1National Institute of Legal Medicine and Forensic Sciences, IP, 2National Institute of Legal Medicine and Forensic Sciences, I.P
Abstract ID 421
Fentanyl abuse in Greece: Presentation of two case reports of fatal intoxication as a result of illegal recreational use with accompanying genetic data.
Orthodoxia Mastrogianni
Antonios Goulas1, Nikolaos Raikos2, Orthodoxia Mastrogianni2, Amvrosios Orfanidis2, Georgios Papazisis3
11st Laboratory of Pharmacology, Faculty of Medicine, Aristotle University of Thessaloniki, 2Laboratory of Forensic Medicine and Toxicology, Faculty of Medicine, Aristotle University of Thessaloniki, 3Laboratory of Clinical Pharmacology, Faculty of Medicine, Aristotle University of Thessaloniki

Abstract ID 423
Comparison of endogenous blood, urine and vitreous humour gamma-hydroxybutyric (GHB) post-mortem concentrations with those from fatal and non-fatal intoxication.
Fiona Perry
Fiona Perry, Lindsey Ward
Eurofins Forensic Services, Toxicology Department

Abstract ID 427
A comparative study of ethyl alcohol concentration in costal cartilage in relation to blood and urine.
Rafal Skowronek
Marcin Tomsiak1, Joanna Nowicka1, El bieta Chelmecka2, Magdalena Wo2, Joanna Wójcik3, Kornelia Droziok1, Rafal Skowronek1
1Department of Forensic Medicine and Forensic Toxicology, Medical University of Silesia in Katowice, 2Department of Statistics, Department of Instrumental Analysis, School of Pharmacy with Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia in Katowice, 3Students Scientific Society, School of Medicine in Katowice, Medical University of Silesia in Katowice

Abstract ID 434
Quantitative analysis of nitrates and nitrites in biological specimens from the suicidal cases by ingestion of sodium nitrite using ion chromatography.
Wonkyung Yang
Wonkyung Yang1, Sanggil Choe2, Sujeong Jeong3, Moonhee Jang4, Hyesun Yum2, Sungmin Moon2
1National Forensic Service, 2National Forensic Service/Seoul Institute

Abstract ID 436
Determination of aluminium and zinc to opine cases of alleged phosphide poisoning using inductively coupled plasma-atomic emission spectrophotometry (ICP-AES).
Anita Yadav1
Anita Yadav1, Adarsh Kumar2, Raj Kumar3, R K Sarin4, Ak Jaiswal5
1All India Institute of Medical Sciences, 2All India Institute of Medical Sciences, New Delhi, 3Forensic Science Laboratory, Madhuban, Karnal, Haryana, 4Forensic Science Laboratory, Vijaywada, andhra Pradesh, 5All India Institute of Medical sciences

Abstract ID 557
Stomach contents as significant investigative tool in postmortem toxicological examinations.
Saima Afzal
Saima Afzal, Humera Shafi, Sara Yasiien, Ali Imran Abid, Muhammad Imran, Mohammad Sarwar, Mohmmad Ashraf Tahir PFSA

Abstract ID 442
Medicolegal autopsies and toxicology analysis.
Hadjazi Omar
Hadjazi Omar, Abdellatif Boublenza, Belhadj Lahcène
University Hospital of Sidi Bel Abbes

Abstract ID 446
Segmental analysis of aripiprazole in postmortem hair from psychiatric patients by use of liquid chromatography–tandem mass spectrometry.
Karen Rygaard
Karen Rygaard1, Jytte Banner2, Kristian Linnet1, Sys Stybe Johansen1
1Section of Forensic Chemistry, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, 2Section of Forensic Pathology, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

Abstract ID 455
Postmortem distribution of paraquat and its metabolites (MP, monoquat) in paraquat poisoning death cases by HPLC-MS/MS.
Keming Yun
Keming Yun1, H Ma2, Y Lu2, L Wang3, Y Wang2, B Huang3, H Zhou2, Z Wei3, S Fu4, Y. Liu2, B Cong2
1Shanxi Medical University, Key Laboratory of Forensic Toxicology, Ministry of Public Security, People’s Republic of China, 2Shanxi Medical University, Key Laboratory of Forensic Toxicology, Ministry of Public Security, People’s Republic of China, 3Shanxi Medical University, Key Laboratory of Forensic Toxicology, Ministry of Public Security, People’s Republic of China, 4University of Technology Sydney, Australia;
Abstract ID 460
The study on post-mortem distribution of carbofuran and its metabolites in rabbits.

Ying Wang
Ying Wang1, Yuping Lu1, Qinglin Guan1, Yuzhen Meng1, Lele Wang1, Zhiwen Wei1, Shanlin Fu2, Yao Liu1, Bin Cong1, Keming Yun1
1. School of Forensic Medicine, Shanxi Medical University, China. 2. Key Laboratory of Forensic Toxicology, Ministry of Public Security, People’s Republic of China.

Abstract ID 461
Study on the stability of EtG and EtS in dried blood spots preserved at different temperatures.

Yuping Lu
Yuping Lu1, Bo Huang1, Ying Wang1, Junkai Li1, Hongjuan Ma1, Hang Zhou1, Zhiwen Wei1, Shanlin Fu2, Yao Liu1, Bin Cong1, Keming Yun1
1. School of Forensic Medicine, Shanxi Medical University, China. Key Laboratory of Forensic Toxicology, Ministry of Public Security, People’s Republic of China.

Abstract ID 462
Analyzing intact carbofuran-7-phenyl glucuronic acid in biological samples by LC-MS/MS.

Ying Wang
Ying Wang1, Yuping Lu1, Hongjuan Ma1, Juan Jia1, Shanlin Fu2, Yao Liu1, Bin Cong1, Zhiwen Wei1, Keming Yun1
1. School of Forensic Medicine, Shanxi Medical University, China. Key Laboratory of Forensic Toxicology, Ministry of Public Security, People’s Republic of China.

Abstract ID 463
Study on the stability of EtG and EtS in human blood preserved at different conditions.

Yuping Lu
Yuping Lu1, Bo Huang1, Ying Wang1, Duo Gong1, Hongjuan Ma1, Lele Wang1, Zhiwen Wei1, Shanlin Fu2, Yao Liu1, Bin Cong1, Keming Yun1
1. School of Forensic Medicine, Shanxi Medical University, China. Key Laboratory of Forensic Toxicology, Ministry of Public Security, People’s Republic of China.

Abstract ID 474
Validation of a Method to Screen for Drugs in Blood and Urine Using Liquid Chromatography Quadrupole Time-of-Flight (LC-QToF) Mass Spectrometry.

Alex Lawson
Alex Lawson, Donna Vincente, Stephen George
University Hospitals Birmingham NHS FT

Abstract ID 483
The postmortem distribution of paraquat and its metabolites (MP, monoquat) in paraquat poisoning death dogs.

Hongjuan Ma
Hongjuan Ma1, Lele Wang2, Ying Wang2, Yu Lu2, Qinglin Guan2, Bo Huang2, Zhiwen Wei2, Shanlin Fu3, Yao Cong3, Bin Cong3, Keming Yun2
1. Shanxi Medical University, 2. Shanxi Medical University; Key Laboratory of Forensic Toxicology, Ministry of Public Security, 3. University of Technology Sydney

Abstract ID 486
Was it drug-induced death? A fatal case with MPHP in the background.

Ewa Tomczak
Ewa Tomczak, Karol Karnecki
Medical University of Gdansk

Abstract ID 487
The toxicokinetics of paraquat and its two metabolites (MP, monoquat) in paraquat poisoning dogs.

Hongjuan Ma
Hongjuan Ma1, Lele Wang2, Ying Wang3, Yuping Lu4, Hang Zhou3, Qinglin Guan3, Zhiwen Wei3, Shanlin Fu3, Yao Cong3, Bin Cong3, Keming Yun3
1. Shanxi Medical University, 2. Shanxi Medical University; Key Laboratory of Forensic Toxicology, Ministry of Public Security, 3. Shanxi Medical University; Key Laboratory of Forensic Toxicology, Ministry of Public Security, 4. University of Technology Sydney.
Abstract ID 497
Contact points of forensic medicine and psychiatry illustrated with fatalities related to new psychoactive substances (NPS).
Malgorzata Klys
Malgorzata Klys, Sebastian Rojek, Karol Kula, Martyna Maciow - Glab, Agnieszka Romanczuk, Kamil Synowiec
Department of Forensic Medicine, Jagiellonian University Medical College

Abstract ID 505
Development of an analytical method to detect pesticides in biological samples of Giant Anteater by liquid chromatography-tandem mass spectrometry (LC-MS/MS).
Mariana Cristina da Silva
Mariana Cristina da Silva¹, Débora Regina Yogui², Mario Henrique Alves², Ana Carolina Furiozo Arantes³, Arnaud Léonard Jean Desbiez⁴, José Luiz da Costa⁴
¹Faculty of Pharmaceutical Sciences, University of Campinas (UNICAMP), Campinas-SP, Brazil; Campinas Poison Control Center, University of Campinas (UNICAMP), Campinas-SP, Brazil, 2Wildlife Conservation Institute (ICAS), Campo Grande - Brazil, 3Faculty of Medical Sciences, University of Campinas (UNICAMP), Campinas-SP, Brazil; Campinas Poison Control Center, University of Campinas (UNICAMP), Campinas-SP, Brazil

Abstract ID 519
Fatal butylone intoxication: a case report.
Vera Maresova
Vera Maresova¹, David Vajtr¹, Alexander Pilin¹, Ludmila Komorousova²
¹Institute of Forensic Medicine and Toxicology, First Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic, ²Institute of Criminalistics, Prague, Czech Republic

Abstract ID 530
Inert Gas Asphyxiation: A Suicide Case Using Industrial Argon in a Household Setting.
Bronwen Davies
University of Cape Town / Western Cape Forensic Pathology Service

Abstract ID 533
The Use of Mortuary Data in Child Death Prevention: The Case of Street Pesticides.
Bronwen Davies
Bronwen Davies¹, Marie Belle Kathrina Auckloo²
¹University of Cape Town / Western Cape Forensic Pathology Service, ²University of Cape Town

Abstract ID 541
Characterization of NPS metabolites through the analysis of hair samples by high-resolution mass spectrometry. A real case of methoxetamine.
José Manuel Matey Cabañas
José Manuel Matey Cabañas¹, Gemma Montalvo García¹, Carmen García Ruiz¹, Maria Dolores Moreno de Simon¹, Adrian Lopez Fernandez¹, Maria Antonia Martinez Gonzalez¹, Gemma Montalvo Garcia¹, Gemma Montalvo Garcia¹, Gemma Montalvo Garcia¹
¹National Institute of Toxicology of Forensic Sciences INTCF, ²Department of Analytical Chemistry, Physical Chemistry and Chemical Engineering and University Institute of Research in Police Sciences (IUICP), ³Department of Chemistry and Drugs, National Institute of Toxicology and Forensic Sciences (INTCF), Ministry of Justice

Abstract ID 546
The first fatality poly-consumption case involving cyclopropyl-fentanyl reported in Madrid.
José Manuel Matey Cabañas
José Manuel Matey Cabañas¹, José Manuel Matey Cabañas¹, Gemma Montalvo Garcia¹, Carmen García Ruiz¹, Juan Carlos Gómez Soro¹, Daniel Gutiérrez Delicado¹, Jovita Rodríguez-Gallardo¹, Maria Antonia Martinez Gonzalez¹
¹National Institute of Toxicology of Forensic Sciences INTCF, ²Department of Chemistry and Drugs, National Institute of Toxicology and Forensic Sciences (INTCF), Ministry of Justice, ³Department of Analytical Chemistry, Physical Chemistry and Chemical Engineering and University Institute of Research in Police Sciences (IUICP), 4Anatomical-Forensic Institute of Madrid
Abstract ID 34
Application of an enantioselective analysis of amphetamine in whole blood in cases of driving under the influence.
Ingrid Bosman
Ingrid Bosman, Lauriane Drouin, Dick-Paul Kloos, Miranda Verschraagen
NFI

Abstract ID 80
Determination of 5-MeO-DIPT in Human Urine Using Gas Chromatography Coupled to high resolution mass spectrometry.
Hui Yan
Hui Yan, Ping Xiang, Xiuying Yan
Academy of Forensic Science, Shanghai Key laboratory of Forensic Medicine, China

Abstract ID 104
Oya Yeter
Oya Yeter, Yeter Erol Öztürk, Tülin Çınar, small Ate
Council of Forensic Medicine

Abstract ID 107
High Data Quality, 24/7 Productivity and Result Defensibility for Blood Alcohol (BAC) determination.
Daniela Cavagnino
Jane Cooper¹, Daniela Cavagnino², Manuela Bergna², Michelle Peace³, Marilyn A. Huestis⁴, Leslie Edinboro⁵
¹Thermo Fisher Scientific, Runcorn, UK, ²Thermo Fisher Scientific, Milan, IT, ³Virginia Commonwealth University, Richmond, VA, USA, ⁴Lambert Center for the Study of Medicinal Cannabis and Hemp, Thomas Jefferson University, Philadelphia, PA, USA, ⁵Quest Diagnostic, US

Abstract ID 131
Urinary concentrations of EtG and EtS after repeated ingestion of ethanol.
Gudrun Høiseth
Gudrun Høiseth¹, Lena Kristoffersen¹, Robert Kronstrand²
¹Oslo University Hospital, ²Rettsmedisinalverket

Abstract ID 184
Quetiapine abuse in city of Adana, Turkey.
Nebile Daglioglu
Nebile Daglioglu, Evsen Guzel, Ismail Ethem Goren
Cukurova University

Abstract ID 208
N-ethylpentylone induced conditioned place preference in rats.
Yulan Rao
Yulan Rao¹, Zebin Lin¹, Hao Wang¹, Zhiru Xu², Yurong Zhang³, Shuqing Zheng³
¹Fudan University, ²China State Institute of Pharmaceutical Industry, ³Shanghai Key Laboratory of Crime Scene Evidence

Abstract ID 219
Blood concentrations of designer benzodiazepines: Findings in forensic cases in Norway.
Gunhild Heide
Gunhild Heide, Gudrun Heiseth, Gerrit Middelkoop, Åse Marit Leere Øiestad
Oslo University Hospital

Abstract ID 259
Development of high-sensitivity method to determination of LSD and 2-oxo-3-hydroxy-LSD in oral fluid by liquid chromatography-tandem mass spectrometry.
Kelly Francisco Cunha
Kelly Francisco Cunha¹, Karina Diniz Oliveira², José Luiz Costa³
¹Department of Pharmacology, Faculty of Medical Sciences, University of Campinas, ²Department of Medical Psychology and Psychiatry, Faculty of Medical Sciences, University of Campinas, ³Faculty of Pharmaceutical Sciences, University of Campinas
Abstract ID 287
Prevalence of therapeutic drugs in blood of injured drivers in Italy. An epidemiological study.
Guido Pelletti
Guido Pelletti1, Marco Garagnani2, Rossella Barone3, Francesca Rossi3, Raffaella Roffi4, Susi Pelotti5, Alain Verstraete6
1University of Bologna, 2Department of Medical and Surgical Sciences, Unit of Legal Medicine, University of Bologna, 3Department of Laboratory Medicine, Ghent University Hospital and department of Diagnostic Sciences, Ghent University

Abstract ID 291
Is the non-linear formation of EtG caused by differences in first pass metabolism of ethanol?
Gudrun Heiseth
Jan Toralf Fosen7, Jørg Merland8, Gudrun Hoiseth9
1Oslo University Hospital, 2Norwegian Institute of Public Health

Abstract ID 299
Serum Ethyl glucuronide as a marker of recent abuse of ethanol in DUI cases - the Italian current situation.
Angelica Capomassi
Angelica Capomassi1, Giovanni Michele Lagravinese2, Tiziana Orzilli2, Gabriella Greco2, Angelica Guidozzi2, Emanuele Guglielmelli2, Sandra Polchi2, Lorenzo Sardone2, Veronica Iorio2, Giuseppe Vinc1, Claudia Mangiapelo3
1La Sapienza University of Rome, 2A.O. San Camillo-Forlanini Rome, 3A.O. San Camillo-Forlanini

Abstract ID 306
A portrait of drugs and driving prior to cannabis legalization in Québec (Canada).
Laurence Paradis-Tanguay
Laurence Paradis-Tanguay, Lucie Vaillancourt, Edith Viel, Cynthia Dombrowski, Brigitte Desharnais, Catherine Lavallée, Pascal Mireault
Laboratoire de sciences judiciaires et de médecine légale

Abstract ID 329
Driving under the influence on cannabis in Portugal (drug level, impairment and result interpretation).
A 8 years retrospective study.
Antonio Castañana
Antonio Castañera, Susana Simões, João Franco, Mário Dias
National Institute of Legal Medicine and Forensic Sciences

Abstract ID 342
Simple and quick determination of 521 psychoactive drugs and their metabolites in human blood, urine and hair samples using LC-MS/MS.
Jakub Czarny
Jakub Czarny1, Michał Raczkowski2, Natalia Galant3, Jadwiga Musial4, Renata Herman5, Magdalena Chrostowska6, Jolanta Powierska-Czarny7, Paulina Jerszy ska8, Barbara Przyjazna9
1Instytut Genetyki Sądowej Sp. z o.o., 2Institute of Forensic Genetics l.l.c., 3Institute of Forensic Genetics Jolanta Powierska-Czarny

Abstract ID 346
Psychoactive substances, their metabolites and drugs occuring in the blood of drivers investigated at the Institute of Forensic Genetics in Bydgoszcz in 2018.
Jadwiga Musial
Jadwiga Musial1, Jakub Czarny2, Natalia Galant3, Michał Raczkowski2, Paulina Jerszy ska2, Jolanta Powierska-Czarny4, Magdalena Chrostowska5, Renata Herman6, Barbara Przyjazna7
1Instytut Genetyki Sądowej Jolanta Powierska-Czarny, 2Institute of Forensic Genetics l.l.c., 3Institute of Forensic Genetics Jolanta Powierska-Czarny

Abstract ID 351
Determination of ethanol in blood samples collected in the central region of Portugal in scope of the Road Code (2017-2018).
Carla Monteiro
Mélanie Pinto1, Alda Claro2, Eugénia Frias2, Proença Paula3, Franco João4, Carla Monteiro5
1Chemistry Department of the Faculty of Sciences and Technologies - University of Coimbra, 2Forensic Chemistry and Toxicology Service; National Institute of Legal Medicine and Forensic Sciences; Portugal.

Abstract ID 353
Alcohol and illicit drug use by victims with severe trauma admitted to the biggest hospital in Latin America in Sao Paulo, Brazil.
Henrique Bombana
Henrique Bombana1, Hallvard Gjerde2, Stig Tore Bogstrand2, Julia Greve1, Vilma Leyton4, Daniel Muñoz4
1University of Sao Paulo, 2Department of Forensic Sciences, Oslo University Hospital, Oslo, Norway, 3Department of Traumatology and Orthopedics, Clinics Hospital, University of Sao Paulo, Sao Paulo, Brazil, 4Department of Legal Medicine, University of Sao Paulo Medical School, Sao Paulo, Brazil
Abstract ID 377
Advancing Forensic DUID Screening with Mass Spectrometry.
Aymeric Morla
Aymeric Morla1, Oscar Cabrices1, Dean Fritch2, Melanie Stauffer2, Nadine Koenig2, Derrick Schollenberger2
1SCIEX, 2Health Network Laboratories

Abstract ID 418
Laura Huppertz
Cynthia Roy1, Kathya LePage1, Pascal Mirault1, Maurice Wilde2, Volker Auwärter2, Laura Huppertz2
1Laboratoire de sciences judiciaires et de médecine légale, Ministère de la Sécurité publique, 1701, rue Parthenais, 12e étage, Montréal (Québec) H2K 3S7, Canada, 2Forensic Toxicology, Institute of Forensic Medicine, Medical Center – University of Freiburg, Albertstraße 9, 19714 Freiburg, Germany

Abstract ID 445
Evaluation of ethanol levels in traffic accidents in Turkey within the scope of legal limits.
Rukiye Döger
Rukiye Döger1, Nihan Tongay2, Serap Annette Akgür2
1Ege University, 2Ege University Institute on Drug Abuse, Toxicology and Pharmaceutical Science

Abstract ID 452
Illicit psychoactive drug use among Brazilian truck drivers.
Henrique Bombana
Juliana Magalhães1, Helena Panizza1, Henrique Bombana1, Daniel Muñoz1, Hallvard Gjerde2, Vilma Leyton1
1University of Sao Paulo, 2Oslo University Hospital

Abstract ID 480
Bio-markers in saliva of Chinese with diazepam ingestion: Diazepam and Nordiazepam.
Lele Wang
Lele Wang1, Xinxin Ren2, Yi He2, Guanfeng Cui2, Hongjuan Ma3, Yaping Lu3, Zhiwen Wei3, Shanlin Fu4, Yao Liu2, Bin Cong5, Keming Yun3
1School of Forensic Medicine, Shanxi Medical University, Key Laboratory of Forensic Toxicology, Ministry of Public Security, People,s Republic of China, 2Institute of Forensic Science, Ministry of Public Security, 3School of Forensic Medicine, Shanxi Medical University, 4University of Technology Sydney, 5Department of Forensic Medicine, Hebei Medical University

Abstract ID 500
Study on the Pharmacokinetics of Alcohol and Its Metabolites in Chinese.
Keming Yun
Keming Yun1, L Wang2, H Ma3, Y Lu2, Y Wang2, Z Wei2, C Zhang2, S Fu3, Y Liu4, B Cong5
1Shanxi Medical University, Key Laboratory of Forensic Toxicology, Ministry of Public Security, People's Republic of China, 2School of Forensic Medicine, Shanxi Medical University, Key Laboratory of Forensic Toxicology, Ministry of Public Security, People's Republic of China, 3University of Technology Sydney, 4Institute of Forensic Science, Ministry of Public Security, 5Department of Forensic Medicine, Hebei Medical University

Abstract ID 516
Decrease of FAEE and EtG in hair by shampoo and water.
Frederike Stoeth
Frederike Stoeth1, Silke Suesse2, Marc Lugnibühl1, Wolfgang Weinmann1
1Institute of Forensic Medicine, University of Bern, Bern, Switzerland, 2DC Drogencheck GmbH, Ulm, Germany
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Abstract ID 10
Simultaneously Identify and Quantify Synthetic Cathinones in Urine Samples by Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry.
Ya-Ling Yeh
Ya-Ling Yeh1, Sheng-Meng Wang2, Chia-Yu Yeh1, Ming-Tsang Yu1
1Criminal Investigation Bureau, 2Central Police University

Abstract ID 16
Fully Automated Forensic Routine Dried Blood Spot Screening for Workplace Testing.
Stefan Gaugler
Stefan Gaugler1, Jana Ryki2, Vicente L. Cebolla3
1CAMAG, 2Shimadzu, 3Instituto de Carboquímica, Miguel Luesma Castán 4., 50018 Zaragoza

Abstract ID 64
Study on distribution of cannabinoids in cannabis plants by desorption electrospray ionization mass spectrometry imaging (II).
Rie Tanaka
Rie Tanaka, Maiko Kawamura, Takashi Hakamatsuka, Ruri Kikura-hanajiri
National Institute of Health Sciences

Abstract ID 66
Quantification of clitidine in caps and stems of poisonous mushroom Paralepistopsis acromelalga by liquid chromatography–tandem mass spectrometry.
Kotaro Hasegawa
Kotaro Hasegawa1, Wurita Amin2, Katsuhiro Konno3, Kimiko Hashimoto4, Kunio Gonmori5, Osamu Suzuki1
1Hamamatsu University School of Medicine, 2Inner Mongolia Medical University, 3University of Toyama, 4Tokyo University of Agriculture

Abstract ID 72
Detection of Synthetic Cannabinoid Metabolites Through Human Liver Microsome Incubation Followed by LC-ESI-Q/TOF Assisted by METABOLITE ID Software.
Sungill Suh
Kyungsoo Oh1, Sang Beom Han1, Hun Young Kim1, Ganganna Bogonda2, Sungill Suh2, Jiyoun Lee1, Chanhee Park1, Hee Seung Kim2, Jin Young Kim1
1Chung-Ang University, 2Supreme Prosecutors’ Office of Korea

Abstract ID 88
Origin of Ethanol in Postmortem Blood: an attempt to Stable Carbon Isotopic Characteristics Values (δ13C) in Forensic Case-work.
Hang Chen
Hang Chen, Sujing Zhang, Baohua Shen, Ping Xiang, Min Shen Academy of forensic science

Abstract ID 114
Methcathinone induces oxidative stress to mediate neurotoxicity through activating apoptosis and autophagy.
Man Liang
Man Liang1, Na Zheng2
1Department of Forensic Medicine, Tongji Medical College, HUST, 2Department of Pathology, School of Basic Medical Sciences, Shen Zhen University

Abstract ID 119
Evaluation of a New Application of Biochip Array Technology to the Simultaneous Screening of Drugs in Hair Samples on the Evidence Investigator Analyser.
V Anderson
V Anderson, L Keery, G Norney, J Darragh, R Walls, M Rodríguez, R McConnell, S Fitzgerald
Randox Toxicology Ltd

Abstract ID 122
Development of a non-target drug screening system in blood using LC–QTOF–MS with in silico approaches
Kanju Saka
Kanju Saka1, Yukiko Nakazono2, Keiko Kudo3, Eishi Imoto4, Tetsuo Iida4, Toshikazu Minohata4, Kazumasa Furuta2, Yusuke Fujii1, Yohsuke Makino1, Hirotarō Iwase1
1Department of Forensic Medicine, Graduate School of Medicine, The University of Tokyo, 2Fujitsu Limited, 3Department of Forensic Medicine and Human Genetics, Kurume University School of Medicine, 4Shimadzu Corporation
Abstract ID 125
Be careful when working in MRM mode: potential false positive results for 2-aminoindane.
Marine Deville
Marine Deville, Nathalie Dubois, Corinne Charlier
Laboratory of Toxicology - CHU Liege

Abstract ID 128
2C-B draws attention on the Belgian market: description of an amphetamine derivative.
Marine Deville
Marine Deville, Corinne Charlier
Laboratory of Toxicology - CHU Liege

Abstract ID 139
Rapid Screening of Alkyl Nitrites by Direct Analysis in Real-Time Time-of-Flight Mass Spectrometry (DART-TOF-MS) with Head-Space Injection.
Ken-ichi Sugie
Ken-ichi Sugie¹, Mamoru Akutsu¹, Koichi Saito²
¹Narcotics Control Department, ²Hoshi University

Abstract ID 144
The Cytotoxicity of the synthetic cannabinoids 5C-AKB48, 5F-MDMB-PINACA, ADB-CHMINACA, MDMBCHMICA and NM-2201 on lung carcinoma and buccal carcinoma cell lines.
Katharina Elisabeth Grafinger
Katharina Elisabeth Grafinger¹, Harpreet Kaur Mandhair², Alain Broillet³, Jürg Gertsch³, Wolfgang Weinmann³
¹Institute of Clinical Pharmacology and Toxicology, ²University of Bern, Institute für Biochemie und Molecular Medicine, National Centre of Competence in Research TransCure, Bern, Switzerland, ³Institute of Forensic Medicine, Forensic Toxicology and Chemistry, University of Bern, Switzerland

Abstract ID 145
Investigating the ability of the microbial model Cunninghamella elegans for the metabolism of synthetic tryptamines.
Katharina Elisabeth Grafinger
Katharina Elisabeth Grafinger¹, Andreas Wilke², Stefan König³, Wolfgang Weinmann³
¹Institute of Clinical Pharmacology and Toxicology, ²Department of Mechanical and Process Engineering, University of Applied Sciences Offenburg, Badstrasse 24, 77652 Offenburg, Germany, ³Institute of Forensic Medicine, Forensic Toxicology and Chemistry, University of Bern, Bern, Switzerland

Abstract ID 149
Development of a novel assay for drugs of abuse based on Molecularly Imprinted Polymers as synthetic antibodies.
Fabiana Grillo
Fabiana Grillo¹, Joanna Czulak², Elena Piletska¹, Michael Whitcombe¹, Sergey Piletsky¹
¹University of Leicester, ²MIP Diagnostics

Abstract ID 154
A novel method of determining tetrodotoxin(TTX) by UPLC-MS/MS applied in two death cases.
Linpei Dong
Linpei Dong, Zhongshan Yu, Xinxin Ren
Institute of Forensic Science, Ministry of Public Security, PR China

Abstract ID 156
Presumptive color tests for design cathinones: Optimization of the test conditions for the novel Neocuproine reagent. Applications as a field test.
Luis Ferrari
Luis Ferrari
UM University

Abstract ID 157
Elucidation of elemental composition and structural properties of the nootropic drugs Picamilon and Aniracetam using LC-HRAM OrbitrapTM MS.
Giampietro Frison
Giampietro Frison, Luca Zamengo, Chiara Bettin, Samuela Frasson, Flavio Zancanaro
Laboratory of Environmental Hygiene and Forensic Toxicology, DMPO Department, AULSS 3
Abstract ID 159
LCMSMS vs GCMS in the detection of COC,EME, cinnamoylcocaine, hygrine and cuscohygrine in the real urine samples of coca leaves chewers after six years storage.
Nelida Cristina Rubio
Nelida Cristina Rubio¹, Antonio Morea-Piñeiro², Ivan Alvarez-Freire³, Pilar Bermejo-Barrera², María Jesús Tabernero-Duque⁴, Sabina Strano-Rossi¹, Ana Maria Bermejo³
¹LATQUIL, ²Department of Analytical Chemistry, Nutrition and Bromatology, Faculty of Chemistry University of Santiago de Compostela, Santiago de Compostela Spain., ³Instituto de Ciencias Forenses “Luis Concheiro” (INCIFOR Forensic Toxicology Service, Forensic Sciences Institute, Medicine School, University of Santiago de Compostela, C/ San Francisco s/n, 15782, Santiago de Compostela, Spain., ⁴Institute of Legal Medicine, Universita` Cattolica del S. Cuore, Rome, Italy.

Abstract ID 165
Application of liquid chromatography-high resolution mass spectrometry (LC-HRMS) to determine male sexual stimulant in selected food matrices.
Ahmad Yusri Mohd Yusop
Ahmad Yusri Mohd Yusop, Linda Xiao, Shanlin Fu
University of Technology Sydney

Abstract ID 167
Development of a non-targeted screening workflow for the detection of synthetic opioids in equine plasma.
Joshua Klingberg
Joshua Klingberg¹, Adam Cawley², Ronald Shimmon¹, Daniel Pasin¹, Chris Fouracre³, Shanlin Fu¹
¹University of Technology Sydney, ²Australian Racing Forensic Laboratory, ³Agilent Technologies

Abstract ID 173
Assessment of concentrations of four phenothiazine antipsychotics in serum and whole blood using different diatomaceous earth-based solid-phase columns.
Takeshi Saito
Takeshi Saito¹, Akira Namera², Tomoatsu Tsuji¹, Sadaki Inokuchi¹
¹Tokai University School of Medicine, ²Hiroshima University

Abstract ID 185
A cautionary tale of targeted LC-MS/MS: the case of an aripiprazole metabolite in urine and TFMPP
Susan Grosse
Susan Grosse, Mark Tyler
Eurofins Forensic Services

Abstract ID 190
In vitro phase I metabolism of the synthetic cannabinoid PX-1 by pooled Human Liver Microsomes and Cunninghamellamella elegans.
Patrick Dahm
Patrick Dahm¹, Andreas Thomas², Markus Alexander Rothschild¹, Mario Thevis², Katja Mercer-Chalmers-Bender¹
¹Institute of Legal Medicine, Faculty of Medicine, University of Cologne, Melatengürtel 60/62, 50823 Cologne, ²Institute of Biochemistry, German Sport University Cologne, Am Sportpark Münzdersorf 6, 50933 Cologne

Abstract ID 191
Chemical characterization of new psychoactive substances belonging to the class of synthetic cathinones in 'legal high' products.
Helena M. Teixeira
João L. Gonçalves¹, Vera L. Alves¹, Maria João Caldeira², José S. Câmara², Helena M. Teixeira³
¹COM - Centro de Química da Madeira, Universidade da Madeira, Campus Universitário da Penteada, 9020-105 Funchal, Portugal, ²Laboratório de Polícia Científica da Polícia Judiciária, Novo edifício-sede da PJ, Rua Gomes Freire 1169-007 Lisboa, ³CQM - Centro de Química da Madeira, Universidade da Madeira, Campus Universitário da Penteada, 9020-105 Funchal, ; Faculdade de Ciências Exactas e da Engenharia, Universidade da Madeira. Campus da Penteada, 9020-105 Funchal, Portugal, ⁴Department of Research, Training and Documentation of the National Institute of Legal Medicine and Forensic Sciences; and Medicine Faculty of the University of Coimbra; Coimbra; Portugal

Abstract ID 190
In vitro phase I metabolism of the synthetic cannabinoid PX-1 by pooled Human Liver Microsomes and Cunninghamellamella elegans.
Patrick Dahm
Patrick Dahm¹, Andreas Thomas², Markus Alexander Rothschild¹, Mario Thevis², Katja Mercer-Chalmers-Bender¹
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João L. Gonçalves1, Vera L. Alves1, Maria João Caldeira2, José S. Câmara3, Helena M. Teixeira4
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Abstract ID 192
Characterization of synthetic cannabinoids in ‘herbal incenses’ products.
Helena M. Teixeira
Vera L. Alves1, João L. Gonçalves1, Maria João Caldeira2, José S. Câmara3, Helena M. Teixeira4
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Abstract ID 195
A Comparative study of Biochip Array Technology versus LC-MS/MS for the screening of NPS and DoA in 161 blood and urine from forensic and clinical casework.
Amine Larabi
Amine LARAB1, Isabelle Etting2, Nicolas Fabresse2, Deborah Cahir3, Victoria Anderson1, Jean-Claude Alvarez2
1Pharmacology-Toxicology Laboratory, 2Pharmacology-Toxicology Laboratory-Garches (France), 3Randox Toxicology, UK

Abstract ID 196
Pharmacokinetics of Methylone and its Metabolites in Male Rats: Relationship to Brain Serotonin Depletion.
Marta Concheiro-Guisan
Marta Concheiro-Guisan1, Nicole Centazzo1, Raider Rodríguez1, Teeshavi Acosta1, Michael Baumann2
1John Jay College, City University of New York, 2National Institute on Drug Abuse, National Institutes of Health

Abstract ID 197
Determination of Methylone and its Three Major Metabolites in Rat Brain by Liquid Chromatography-Mass Spectrometry.
Nicole Centazzo
Marta Concheiro-Guisan, Raider Rodriguez, Nicole Centazzo
John Jay College, City University of New York

Abstract ID 216
Quantification of ethanol in whole blood by extraction using NeedlEx® and gas chromatography/mass spectrometry.
Takayoshi Suzuki
Takayoshi Suzuki1, Masae Iwai1, Fumio Kondo2, Tadashi Ogawa1, Hiroshi Seno1
1Department of Legal Medicine, Aichi Medical University School of Medicine, 2Department of Biomedical Sciences, Chubu University College of Life and Health Sciences

Abstract ID 229
Determination of ofcentanyl and W-18 in a suspicious heroin-like powder in Belgium.
Maarten Degrefe
Maarten Degrefe1, Peter Blankaert2, Eleanor M. Berry1, Alexander L.N. van Nuijs1, Kristof E. Maudens3
1Toxicological Centre - University of Antwerp, 2Belgian Early Warning System on Drugs - Drugs Program Sciensano, 3Eurofins Forensics Belgium

Abstract ID 242
Metabolomic analysis of different cannabis species by untargeted HPLC-HRMS.
Christoph Hassenberg
Christoph Hassenberg1, Katja Mercer-Chalmers-Bender2, Justus Beike1, Jennifer Schürenkamp1
1Institute of Legal Medicine, University Hospital Münster, 2Institute of Forensic Medicine, University of Basel, Health Department Basel-Stadt

Abstract ID 243
Assessment of biased agonism amongst distinct synthetic cannabinoid receptor agonists scaffolds.
Christophe Stove
Elise Wouters1, Max Meyrath2, Martyna Szpakowska2, Andy Chevigné2, Christophe Stove1
1Ghent University, 2Luxembourg Institute of Health
Abstract ID 245
Application of a mu opioid receptor bioassay platform to study biased signalling of novel synthetic opioids.
Christophe Stove
Lakshmi Vasudevan1, Marie Deventer2, Elise Wouters2, Annelies Cannaert2, Max Meyrath3, Martyna Szpakowska2, Andy Chevigne3, Deepak Saini4, Christophe Stove2
1Ghent University, 2Laboratory of Toxicology, Department of Bioanalysis, Faculty of Pharmaceutical Sciences, Ghent University, Belgium, 3Immuno-Pharmacology and Interactomics, Infectious Diseases Research Unit, Department of Infection and Immunity, Luxembourg Institute of Health, Luxembourg, 4Department of Molecular Reproduction, Development and Genetics, Indian Institute of Science, Bangalore, India

Abstract ID 247
SherLOK: Using life-style markers for perpetrator profiling through untargeted screening of hair.
Ana Miguel Pego
Ana Miguel Pego1, Edward Knaven1, Anke van de Plas1, Arian van Asten1, Ben de Rooij1, Theo Noij1
1Research group Analysis Techniques in the Life Sciences, Avans University of Applied Sciences, 2Co van Ledden Hulsebosch Center, University of Amsterdam

Abstract ID 250
Development and validation of a GC-MS methodology for the determination of opioids in whole blood and pericardial fluid: Application to authentic specimens.
Cláudia Margalho
Elisa Ferreira, Alice Castanheira, João Franco, Francisco Corte-Real, Cláudia Margalho
National Institute of Legal Medicine and Forensic Sciences

Abstract ID 271
Cocaine, benzoylecgonine and cuscohygrine profile in plasma from a volunteer who chews coca leaves. Preliminary study.
Nelida Cristina Rubio
Nelida Cristina Rubio1, Antonio Moreda-Piñeiro2, Ivan Álvarez-Freire3, Pilar Bermejo-Barrera2, Maria Jesús Tabernero-Duque3, Ana Maria Bermejo1
1LATÓQUILO, 2Department of Analytical Chemistry, Nutrition and Bromatology, Faculty of Chemistry University of Santiago de Compostela, Santiago de Compostela Spain., 3Instituto de Ciencias Forenses "Luis Concheiro" (INCIFORE Forensic Toxicology Service, Forensic Sciences Institute, Medicine School, University of Santiago de Compostela, C/ San Francisco s/n, 15782, Santiago de Compostela, Spain.

Abstract ID 281
Hydrolytic stability of 32 synthetic cannabinoids with valine- and tert-leucine methyl ester or amide as linked group in blood serum and cardiac blood samples.
Sebastian Halter
Sebastian Halter, Volker Auwärter
Institute of Forensic Medicine - Forensic Toxicology

Abstract ID 289
Risk and Opportunity Assessment System - Probability and Consequences Method.
Carla Monteiro
Pedro Costa, Lara Sousa, M. José Quintas, Paula Melo, João Franco, Carla Monteiro
Forensic Chemistry and Toxicology Service; National Institute of Legal Medicine and Forensic Sciences; Portugal.

Abstract ID 294
Analytical challenges in the forensic toxicological analysis of a subgroup of novel synthetic opioids, the “U-drugs”.
Maurice Wilde
Maurice Wilde1, Volker Auwärter2
1Institute of Forensic Medicine - Forensic Toxicology, 2Forensic Toxicology, Institute of Forensic Medicine, Medical Center – University of Freiburg, Freiburg, Germany

Abstract ID 295
New Findings on Type and Amount of Tryptamine Derivatives in the Poison of the Colorado River Toad (Incilius alvarius) using LC-HR-QTOF-MS and LC-MS/MS.
Merja A. Neukamm
Nicole Zimmermann1, Tobias Scholl3, Johannes Penner3, Amy Autret4, Thomas Zander2, Laura M. Huppertz1, Volker Auwärter3, Merja A. Neukamm1
1Medical Center - University of Freiburg, 2ESA-Test GmbH, 3Chair of Wildlife Ecology & Management Freiburg, 4Crime Laboratory Tucson Police Department
Abstract ID 310
A non-destructive and versatile approach for the detection of psychoactive substances by X-ray powder diffraction
Bronislav Jurasek
Bronislav Jurasek, Stepan Huber, Alzbeta Nemeskalova, Katerina Hajkova, Frantisek Kralik, Vilem Bartunek, Martin Kuchar
University of Chemistry and Technology, Prague

Abstract ID 312
Drugs of Abuse Screening in Urine and Quantification from Whole Blood using PaperSpray Technology for Forensic Use.
Valérie Thibert
Valérie Thibert, Neloni Wijeratne, Cornelia Boeser
Thermo Fisher Scientific

Abstract ID 313
UPLC-MS/MS analysis of illicit drugs in wastewater in the city of Lisbon and Almada between 2015 – 2018. Cross correlations with major nicotine biomarker.
Susana Simões
Susana Simões¹, Álvaro Lopes², João Franco¹, Mário Dias¹
¹National Institute of Legal Medicine and Forensic Sciences, ²Faculty of Pharmacy – University of Lisbon

Abstract ID 317
Method development for the identification of several mycotoxins by laser desorption – mass spectrometry (LDTD-Orbitrap).
Estelle Flament
Estelle Flament¹, Jérôme Guitton², Yvan Gaillard1, Serge Auger3, Jean-Michel Gaulier4
¹LAT LUMTOX, ²Criblement thérapeutique en Oncologie, UFR Faculté de Médecine Lyon-Sud, ³Phytronic, ⁴IMPECS, IMpact de l’Environnement Chimique sur la Santé humaine, Université de Lille

Abstract ID 325
In search of psychoactive THC in cannabis-based dietary supplements.
Alzbeta Nemeskalova
Alzbeta Nemeskalova¹, Katerina Hajkova², Bronislav Jurasek², Lukas Mikulu², David Sykora², Martin Kuchar²
¹University of chemistry and technology in Prague, ²University of chemistry and technology Prague

Abstract ID 338
Determination of Synthetic Cannabinoids Usage Frequency in Probationers.
Aslı Atasoy
Aslı Atasoy¹, Nebile Da Io Lu², Serap Annette Akgür³
¹Cukurova University, Institute of Addiction and Forensic Sciences, ²Cukurova University, Medicine Faculty, Department of Forensic Medicine, ³Ege University, Institute on Drug Abuse, Toxicology and Pharmaceutical Science

Abstract ID 341
Differentiation of blood spots originating from uniovular twins based on xenobiotics analysis.
Jakub Czarny
Jakub Czarny¹, Joanna Idkowiak², Monika Antczak², Aleksandra Górka², Łukasz Kaczorowski², Bartosz Hornik²
¹Instytut Genetyki Sądowej Sp. z o.o., ²Institute of Forensic Genetics l.l.c.

Abstract ID 352
Analytical workflow in the era of the opioids crisis: screening for novel synthetic opioids.
Béatrice Garneau
Béatrice Garneau¹, Brigitte Desharmais², Pascal Mireault², André Lajeunesse¹
¹Université du Québec à Trois-Rivières, ²Laboratoire de sciences judiciaires et de médecine légale

Abstract ID 356
Alicyclic fentanyl’s µ-opioid receptor activation changes from potent full agonists to partial agonists with increasing ring structure.
Anna Åstrand
Anna Åstrand¹, Ingrid Jakobssen¹, Niclas Björn¹, Svante Vikingsson², Robert Kronstrand², Henrik Grén²
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Detection and phase I metabolism of the 7-azaindole derived synthetic cannabinoid 5F-AB-P7AICA including a preliminary pharmacokinetic evaluation.
Arianna Giorgetti
Arianna Giorgetti¹, Lukas Mogler², Belal Haschimi², Sebastian Halter², Florian Franz², Westphal Folker², Svenja Fischmann³, Volker Auwärter²
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Prevalence estimation of synthetic cannabinoid use in prisons and forensic psychiatric hospitals before and after the introduction of the NpSG.
Julia Kaudewitz
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Ultra-Sensitive Forensic Analysis of Cocaine and its Metabolites in Hair Samples.
Aymeric Morla
Aymeric Morla¹, Janna Anichina¹, Oscar Cabrices¹, Pierre Negri¹, Sean Orlowicz², Laura Snow²
¹SCIEX, ²Phenomenex

Rapid and low-cost determination of Carbohydrate-Deficient Transferrin (CDT) based on Fluorescence Resonance Energy Transfer (FRET).
Giacomo Musile
Giacomo Musile¹, Elio F De Palo², Sergey A Savchuk³, Kseniia Shestakova³, Federica Bortolotti³, Franco Tagliaro⁵
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Quantitative Analysis of Fentanyl and Analogues in Human Whole Blood.
Aymeric Morla
Aymeric Morla¹, Casey Burrows¹, Alex Krotulski², Diana Tran¹, Kevin He¹, Alex Wang¹
¹SCIEX, ²Center for Forensic Science Research and Education at the Fredric Rieders Family Foundation

Characterization of 4-chloroethcathinone by GC-MS after 2,2,2-trichloroethyl chloroformate derivatization, LC-HRAM Orbitrap™ MS, and solid deposition GC-FTIR.
Giampietro Frison
Luca Zamengo¹, Giampietro Frison¹, Chiara Bettin¹, Flavio Zancanaro¹, Emanuela Trovato², Luigi Mondello²
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Differentiation of the isomers isomephedrone and mephedrone by GC-MS after 2,2,2-trichloroethyl chloroformate derivatization, LC-HRAM Orbitrap™ MS, and GC-FTIR.
Giampietro Frison
Giampietro Frison¹, Luca Zamengo², Gianpaola Tedeschi², Emanuela Trovato³, Tania Salerno³, Luigi Mondello³
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Studies in Fentanyl Metabolism for Forensic Toxicology: Analysis of Fentanyl and Metabolites Following the Medical Administration of Fentanyl.
Michelle Wood
Jonathan Danaceau¹, Michelle Wood², Melissa Ehlers³, Thomas G Rosano⁴
¹Waters Corporation, Milford, MA, USA, ²Waters Corporation, Wilmslow, UK, ³Albany Medical Center, Albany, NY, USA, ⁴National Toxicology Center, Albany, NY, USA
Abstract ID  395
Evaluation of DART QDa - an Ambient Ionization Technique Coupled with a Mass Detector for Rapid Forensic Drug Screening.

Nayan S Mistry
Michelle Wood¹, Nayan S Mistry²
¹Waters Corp, ²Waters Corporation, Wilmslow, UK

Abstract ID  399
Non-target screening method for drugs in blood by combinational use of LC/Q-TOFMS and Micro Volume QuEChERS kit.

Yujin Natori
Yujin Natori¹, Eishi Imoto², Kengo Matsumoto¹, Mikael Levi², Hitoshi Tsuichihashi¹, Akira Ishii¹, Tairo Ogura², Kei Zaitsu³
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Abstract ID  404
Online µSPE for fully automated LC-MS screening of urine samples in forensic and clinical toxicology.

Michaela Schmidt
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Abstract ID  407
Establishment of extraction procedure using ISOLUTE PLD+ protein and phospholipid removal column for LC-MS/MS analysis of 20 psychoactive drugs in whole blood.

Tadashi Ogawa
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¹Department of Legal Medicine, Aichi Medical University School of Medicine, ²Department of Oral Pathology, Aichi Gakuin University School of Dentistry, ³Department of Biomedical Sciences, Chubu University College of Life and Health Sciences

Abstract ID  410
Determination of Time since Deposition (TsD) of biological crime scene traces by mass-spectrometry based proteomics.

Tom Dario Schneider
Tom Dario Schneider¹, Jonas Grossmann², Bernd Roschitzki², Nathalie Selevec³, Thomas Kraemer³, Andrea Eva Steuer³
¹Zurich Institute of Forensic Medicine, ²Functional Genomics Centre Zurich (FGCZ), University of Zurich/ETH Zurich, Switzerland, ³Zurich Institute of Forensic Medicine (ZIFM), University of Zurich, Switzerland

Abstract ID  411
Recommendations for the determination of matrix suppression in biological samples by UPLC-ESI-MS/MS: Extending Quality Measures in Forensic Toxicology.

Sabra Botch-Jones
Sabra Botch-Jones¹, Courtney McGowan², Jamie Foss³, Kacey Cliburn⁴, Sabra Botch-Jones⁵
¹Boston University School of Medicine, ²Biomedical Forensic Sciences, Boston University School of Medicine, ³PerkinElmer, ⁴Civil Aerospace Medical Institute, Federal Aviation Administration, ⁵Boston University School of Medicine, Biomedical Forensics

Abstract ID  412
Metabolism of isobutyrylfentanyl modelled by human hepatocytes and analyzed by LC-QTOF-MS.

Shimpei Watanabe
Shimpei Watanabe¹, Henrik Lindblom², Anna Åstrand², Svante Vikingsson¹, Robert Kronstrand³, Henrik Gréen³
¹National Board of Forensic Medicine, ²Linköping University, ³National Board of Forensic Medicine & Linköping University

Abstract ID  413
Synthesis, determination of metabolites and in vitro cytotoxicity of deschloroketamine enantiomers.

Martin Kuchar
Martin Kuchar¹, Katerina Hajkova², Bronislav Jurasek², Silvie Rimpelova², Tomas Palenicek³, Michal Kohout⁴, Dita Spalovska⁶
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Abstract ID 416
Paper-based detection of 25-NBOMe compounds in oral fluid.
Laura Clancy
Laura Clancy, Ronald Shimmon, Shanlin Fu
University of Technology Sydney

Abstract ID 435
Comparison of internal standards for determining glyphosate, glufosinate and their metabolites in human Plasma by LC-MS/MS.
Akira Ishii
Akira Ishii, Kei Zaitsu, Yujin Natori, Hitoshi Tsuchihashi
Department of Legal Medicine and Bioethics, Nagoya University Graduate School of Medicine

Abstract ID 439
The role of formaldehyde in the mechanisms of acute methanol intoxication.
David Sykora
David Sykora¹, Vladimir Kral², Sergej Zacharov³
¹Department of Analytical Chemistry, University of Chemistry and Technology, Prague, Czech Republic, ²Department of Analytical Chemistry, University of Chemistry and Technology Prague, Prague, Czech Republic, ³Department of Occupational Medicine, 1st Faculty of Medicine, Charles University, Prague, Czech Republic

Abstract ID 447
NPSs analysis in biological samples: from GC-MS to LC-HRMS.
Sara Odoardi
Sara Odoard³, Serena Mestria¹, Valeria Valentini¹, Sofia Federici¹, Gilbert Mercieca², Sabina Strano Rossi¹
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Abstract ID 453
Laminar Flow Mass Spectrometry for the Detection and Quantification of Fentanyl, Norfentanyl, and Acetaminophen in Whole Blood.
Hajin Hwang
Hajin Hwang¹, Jenna Gardner¹, Boyle Sarah¹, Jaime Foss¹, Collin Hill², Frank Kero², Karen Scott³, Sabra Botch-Jones¹
¹Boston University School of Medicine, ²PerkinElmer, ³Arcadia University

Abstract ID 468
Evaluation of Sample Preparation Approaches for the Extraction of Amphetamine, Methamphetamine, MDMA and metabolites from Urine prior to GC/MS Analysis.
Adam Senior
Lee Williams, Katie-Jo Teehan, Adam Senior, Helen Lodder, Geoff Davies, Alan Edgington, Claire Desbrow, Paul Roberts, Rhys Jones
Biotage GB Limited

Abstract ID 470
Comparison of Mid-InfraRed, Near-InfraRed and Raman spectroscopy combined with chemometrics to classify and quantify seized cocaine powders.
Nele Samyn
Joy Elaerts¹, Natalie Meert², Pierre Dardenne³, Vincent Baeten³, Filip Van Durme³, Nele Samyn³, Karolien De Wael⁴
¹NICC, ²National Institute of Criminalistics and Criminology (NICC), Department of Toxicology and Drugs, ³Walloon Agricultural Research Centre (CRA-W), ⁴University of Antwerp, Chemistry Department, AXES Research Group

Abstract ID 481
Detection of drugs of abuse in DBS and DUS collected on a dried matrix spot cardboard cartridge using on-line SPE-HPLC-MS/MS.
Tim Johan Gelmi
Tim Johan Gelmi¹, Lot Claeys², Adiamantis Krokos³, Katharina Grafinger¹, Nikos Raikos³, Wolfgang Weinmann¹
¹Institute of Forensic Medicine Bern, ²Ghent University, ³Aristotle University of Thessaloniki, ⁴University Hospital Bern

Abstract ID 482
A Benchtop NMR approach for the determination of ethanol and methanol in alcoholic beverages in cases of suspected poisonings and drink spiking.
Geraldine Dowling
Geraldine Dowling¹, Pierce Kavanagh², Juan Araneda³, Susie Riegel³, Oliver Joyce⁴, Simon Brandt⁵
¹Institute of Technology Sligo, ²Trinity College Dublin, ³Navanalyis, ⁴IT Sligo, ⁵Liverpool John Moores University
Abstract ID 485
Phase I metabolic profiling of the synthetic cannabinoid EG-018 by pooled human liver microsomes, CYP isoenzymes, Cunninghamella elegans and urine screening.
Franziska Gaunitz
Franziska Gaunitz1, Patrick Dahm1, Lukas Mogler1,2, Andreas Thomas4, Mario Thevis4, Katja Mercer-Chalmers-Bender1,5
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Abstract ID 489
Analysis of diazepam and its metabolites in dried blood spots using HPLC–MS/MS.
Lele Wang
Lele Wang1, Xuezhi Wang2, Hongjuan Ma2, Yiping Lu2, Ying Wang2, Zhiwen Wei2, Chao Zhang2, Shanlin Fu2, Yao Liu4, Bin Cong2, Keming Yun1
1School of Forensic Medicine, Shanxi Medical University, Key Laboratory of Forensic Toxicology, Ministry of Public Security, People’s Republic of China, 2School of Forensic Medicine, Shanxi Medical University, Key Laboratory of Forensic Toxicology, Ministry of Public Security, People’s Republic of China, 3University of Technology Sydney, 4Institute of Forensic Science, Ministry of Public Security, 5Department of Forensic Medicine, Hebei Medical University

Abstract ID 496
Drug screening in the DUID context by using the ToxtyperTMLC-MSn: preliminary evaluation of its application to serum samples.
Rossella Gottardo
Rossella Gottardo1, Matilde Murari1, Anna Bertaso1, Federica Bortolotti1, Franco Tagliaro2
1Dept. of Diagnostics and Public Health - University of Verona, 2Dept. of Diagnostics and Public Health - University of Verona - Institute Translational Medicine and Biotechnology, Sechenov First Moscow State Medical University, Moscow, Russian Federation

Abstract ID 503
Abused drugs, heavy metals and microbiological analysis in Izmir Kucuk Menderes river.
Yeşim Karabulut
Yeşim Karabulut1, Rukiye Döer2, Melike Aydo du2, Serap Annette Akgür2
1Ege University, 2Ege University, Institute on Drug Abuse, Toxicology and Pharmaceutical Science (BATI)

Abstract ID 504
Solid deposition GC-FTIR and LC-FTIR as highly discriminating techniques for forensic toxicology applications.
Tania Salerno
Tania Salerno1, Paola Donato1, Giampietro Frison2, Margita Utczas2, Emanuela Trovato2, Luigi Mondello3
1BeSep Srl c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, 2Department of Biomedical, Dental, Morphological and Functional Imaging Sciences, University of Messina, 3Laboratory of Environmental Hygiene and Forensic Toxicology, DMPO Department, AULSS 3, Venice, Italy, 4University of Physical Education, Budapest, Hungary, 5Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy, 6Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy

Abstract ID 510
The Qualitative and Quantitative Analysis of Synthetic Cannabinoid Receptor Agonists in Infused Papers within Prison Settings - A Temporal Study 2018-2019.
Caitlyn Norman
Caitlyn Norman1, Ciara MacDonald2, Gillian Walker3, Brian McKirdy3, Niamh Nic Daeid4, Craig McKenzie1
1Forensic Drug Research Group, Centre for Anatomy and Human Identification, University of Dundee, 2University of Strathclyde, 3Scottish Prison Service, 4Leverhulme Research Centre for Forensic Science, University of Dundee

Abstract ID 522
Development a chemometric method in urine for new designer psychoactive hallucinating substance: 5-MEO-MiPT.
Eziğ Emen
Eziğ Emen1, Rukiye Dö er2, Melike Aydo du2, Halil brahim Bostancı2, Hasan Erta 3, Serap Annette Akgür2
1Ege University, Institute on Drug Abuse, Toxicology and Pharmaceutical Science, 2Ege University

Abstract ID 524
Analysis of the main components of ayahuasca and their cytotoxicity in dopaminergic cells.
Jose Restolho
Ana Simão1, Joana Gonçalves1, Débora Caramelo1, Tiago Rosado1, Mário Barroso2, Jose Restolho3, Nicolás Fernández4, Ana Paula Duarte4, Ana Clara Cristóvão5, Eugénia Gallardo1
1Centro de Investigação em Ciências da Saúde, Universidade da Beira Interior (CICS-UBI), 2Servicio de Química e Toxicologia Forenses, Instituto de Medicina Legal e Ciências Forenses - Delegação do Sul, 3nal von minden GmbH, 4Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, 5NEUROSOV, UBImedical, Universidade da Beira Interior
Abstract ID 526
Detection and Quantitation of Cannabidiol and D(9)-Tetrahydrocannabinol in Oral Fluid of a Therapeutic-Use Cannabidiol Donor Using UHPLC-Laminar Flow-MS/MS

Jenna Gardner
Jenna Gardner1, Hajin Hwang1, Sarah Boyle1, Collin Hill2, Mikayla Caldwell1, Sabra Botch-Jones1
1Boston University School of Medicine, 2PerkinElmer

Abstract ID 545
A low voltage paper spray ionization Q-TOF method for screening of NPS in street drug blotter samples

Letícia Birk
Letícia Birk1, Sarah Eller Franco de Oliveira1, Gabriela Mafra2, Rafael Brognoli2, Marcos José Souza Carpes3, Daniel Scolmeister3, Eduardo Carasek2, Josias Merib1, Tiago Franco de Oliveira1
1Federal University of Health Sciences of Porto Alegre, Brazil, 2Federal University of Santa Catarina, Brazil, 3Instituto Geral de Perícias - IGP/RS, Brazil
Abstract ID 138
Rapid detection of anabolic doping agents in non-invasive biological matrices using nanomaterial based SALDI-MS.
Rajpreet Minhas
Rajpreet Minhas, Hashim Al Hmoud, David Rudd, Nicolas Voelcker
Monash University

Abstract ID 178
Detection of synthetic peptides in dried blood spots by means of UHPLC-HRMS: a proof of concept for anti-doping analysis.
Enrico Gerace
Enrico Gerace¹, Jessica Modaffari², Daniele Di Corcia¹, Marco Vincenti², Alberto Salomone³
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Abstract ID 262
Optimization of a method for the detection of anabolic androgenic steroids in dried urine spots by paper spray-mass spectrometry ionization.
Ana Luiza Freitas de Assis Linhares
Ana Luiza Freitas de Assis Linhares¹, Francis Ribeiro de Souza², Maria Janieire de Nazaré Nunes Alves², Yuri Machado³, Rogério Araújo Lordeiro³, Mauricio Yonamine¹
¹Faculty of Pharmaceutical Sciences, University of Sao Paulo, ²Heart Institute, Faculty of Medicine of the University of Sao Paulo, ³Institute of Criminalistics, Civil Police of Minas Gerais

Abstract ID 307
Mass spectrometric characterization of Melanotan II, a skin pigmentation enhancer sold on the black market of PIEDs.
Sara Odoardi
Sara Odoardi¹, Serena Mestria¹, Giampietro Frison², Sabina Strano Rossi¹
¹Forensic Toxicology Laboratory, Institute of Public Health, Università Cattolica del Sacro Cuore, Rome, Italy, ²Laboratory of Environmental Hygiene and Forensic Toxicology, DMPO Department, AULSS 3, Venice, Italy

Abstract ID 401
Undisputable results by coupling of GC-IRMS with high-resolution mass spectrometry for final confirmation in sports drug testing.
Julian Renpenning
Julian Renpenning, Mario Tuthorn, Dieter Juchelka
Thermo Fisher Scientific

Abstract ID 454
Analysis of seizures formulations of stanozolol in South Brazil by High Resolution Mass Spectrometry.
Sarah Eller Franco de Oliveira
Everton Campos, Leticia Birk, Sandra Macedo, Josias Merib, Sarah Eller Franco de Oliveira, Tiago Oliveira
Federal University of Health Sciences of Porto Alegre

Abstract ID 506
The working horse Q-exactive Focus, 2 years of screening doping control samples.
Kim Pettersson-Bohlin
Kim Pettersson-Bohlin, Carmel Heiland, Magnus Ericsson
Doping Control Laboratory
Abstract ID 15
High-throughput determination of tegafur and 5-fluorouracil in human tear and plasma by HILIC-MS/MS.
Xiao-Pen Lee (Akihiro Nakauchi)
Xiao-Pen Lee (Akihiro Nakauchi)1, Ritsuko Shiokawa2, Masaya Fujishiro3, Takeshi Kumazawa4, Chika Hasegawa5, Hiroshi Sakamaki6, Shigehiro Iwabuchi7, Hidetoshi Onda8, Keizo Sato9, Takaaki Matsuyama10
1Department of Legal Medicine, Showa University School of Medicine, 2Department of Ophthalmology, Showa University School of Medicine, Japan, 3Department of Legal Medicine, Showa University School of Medicine, Japan, 4Seirei Christopher University School of Nursing, Japan, 5Department of Legal Medicine, Toho University School of Medicine, Japan, 6Chemicals Evaluation and Research Institute, Japan

Abstract ID 28
Prediction of Synthetic Cannabinoids LC Retention by Quantitative Structure-Property Relationship as an aid to identification of new/unknown compounds.
Aldo Eliano Polettini
Aldo Eliano Polettini1,2, Johannes Kutzler2, Christoph Sauer2, Sergej Bleicher2, Wolfgang Schultis2
1Department of Diagnostics & Public Health, University of Verona, 2Department of Toxicology and Forensic Toxicology, Synlab MVZ Weiden GmbH, Weiden, Germany

Abstract ID 35
Prenatal exposure to recreational drugs and alcohol in neonates admitted to a neonatal intensive care unit.
Bernardino Barcelo
Bernardino Barcelo1, Antonia Roca2, Pilar Jarque2, Isabel Gomila3, Sara Malaca1, Emilia Marchei4, Miguel Angel Elorza5
1Clinical Analysis Department, Hospital Universitari Son Espases, Research Institute of Health Sciences (IdISBa), Palma de Mallorca, Spain, 2Division of Neonatology, Department of Paediatrics, University Hospital Son Espases, Palma de Mallorca, Spain, 3Clinical Analysis Department, Hospital Universitari Son Llàtzer, Research Institute of Health Sciences (IdISBa), Palma de Mallorca, Spain, 4Centro de Investigação em Ciências da Saúde (CICS-UBI), Laboratório de Fármaco-Toxicologia, Universidade da Beira Interior, Covilhã, Portugal, 5National Centre on Addiction and Doping, Istituto Superiore di Sanità, Rome, Italy, 6Clinical Analysis Department, Hospital Universitari Son Espases, Palma de Mallorca, Spain

Abstract ID 58
Sensitive liquid chromatography/tandem mass spectrometry method for the simultaneous determination of thirteen beta blockers.
Keiko Tonooka (Kubota)
Keiko Tonooka (Kubota), Masaru Terada, Tetsushi Hosono, Keita Takanashi, Tatsu Shinozuka
Yokohama University of Pharmacy

Abstract ID 69
Benzodiazepines facilitate intoxication to oxycodone: evidence of metabolic interaction with diazepam and a designer drug, diclazepam.
Franck Saint-Marcoux
Franck Saint-Marcoux1, Roland Lawson1, Franck Saint-Marcoux2, Souleiman El Balki3
1Department of pharmacology and toxicology, 2University Limoges - Inserm U1248, 3Department of pharmacology and toxicology, Limoges University hospital

Abstract ID 75
Stability of Amphetamines in Dried Blood Spots by using GC Methods.
Huda Alkhalaf
Huda Alkhalaf1, Khalid Masoud2, Mohammed Elramady2
1Prince Sultan Military Meical City, 2Naif Arab University for Security Science

Abstract ID 76
Phenethylamine-derived new psychoactive substances: metabolic fate and toxicological detectability of three 2C-FLY drugs.
Lea Wagmann
Lea Wagmann1, Lea Wagmann2, Nora Hempel1, Lilian H.J. Richter1, Simon D. Brandt2, Alexander Stratford3, Markus R. Meyer2
1Saarland University, 2Department of Experimental and Clinical Toxicology, Saarland University, Germany, 3School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool, UK, 4Synex Synthetics BV, Maastricht, The Netherlands
Abstract ID 77
In vivo and In vitro investigations on the metabolic fate of N-ethyl-N-propyl-tryptamine, 2-aminoindane, and N-methyl-2-aminoindane.
Sascha Manier
Sascha Manier1, Christina Felske1, Niels Eckstein2, Markus R. Meyer1
1Saarland University, 2University of Applied Sciences Kaiserslautern

Abstract ID 94
Comparison of the detection windows of heroin metabolites in human urine using online SPE and LC-MS/MS: importance of morphine-3-glucuronide.
Yurong Zhang
Yurong Zhang1, Luying Wang2, Chen Liang1, Rong Wang1, Kuadou Wang1, Fangqi Cao3
1Shanghai Key Laboratory of Crime Scene Evidence, Shanghai Institute of Forensic Science, 2Shanghai Key Laboratory of Crime Scene Evidence, Shanghai Institute of Forensic Science/State Key Laboratory of New Drug and Pharmaceutical Process, China State Institute of Pharmaceutical Industry, 3Shanghai Key Laboratory of Crime Scene Evidence, Shanghai Research Institute of Criminal Science and Technology

Abstract ID 115
Measurement of antipsychotic medications and evaluation of drug melanin affinity in head hair samples from criminal justice population.
Maria del Mar Ramirez Fernandez
Maria del Mar Ramirez Fernandez1, Werner A. Baumgartner2, Sarah. M.R. Wille1, Nele Samyn1
1National Institut of Criminalistics and Criminology, 2Ianus Foundation

Abstract ID 130
Bogumila Byrska
Bogumila Byrsa1, Dariusz Zuba1, Roman Stanaszek2, Wioleta Wrzesie2
1Institute of Forensic Research, 2Institute of Forensic Science

Abstract ID 133
Hystotoxicological Examination of A New Design Psychoactive Substance 5-MeO-MiPT.
Melike Aydoğdu
Melike Aydo du1, Melike Aydo du1, Yusuf Ali Altunci1, Eda Açıkl göz1, Ümmü Güven1, Fahriye Düz a aç1, Asli Atasoy2, Neble Da io lu2, Gülperi Öktem1, Serap Annette Akgür1
1Ege University, 2Çukurova University

Abstract ID 147
Studies on the in Vitro and in Vivo Metabolism of the New Synthetic Opioids U-51754, U-47931E, and Methoxyacetylfentanyl Using LC-High Resolution-MS/MS.
Frederike Nordmeier
Frederike Nordmeier1, Lilian H. Richter2, Peter H. Schmidt1, Nadine Schaefer1, Markus R. Meyer2
1Institute of Legal Medicine, Saarland University, 2Department of Experimental and Clinical Toxicology, Saarland University

Abstract ID 158
Massive colchicine poisoning, when outcome is not fatal.
Anne-Sophie Lemaire-Hurtel
Anne-Sophie Lemaire-Hurtel1, Charlotte Quinton-Bouvier1, Jean-Claude Alvarez2, Julien Maizel1, Sandra Bodeau1, Youssef Bennis1
1CHU AMIENS, 2CHU GARCHES

Abstract ID 168
Evaluation of micro volume sample preparation technology newly designed for forensic toxicology with High Resolution Accurate Mass Spectrometry.
Mikael Levi
Eishi Imoto1, Mikael Levi1, Yujin Nator2, Jun Watanabe1, Hotishi Tsuchihachi2, Kei Zaitsu2
1Shimadzu Corp., 2Department of Legal Medicine & Bioethics, Nagoya University Graduate School of Medicine

Abstract ID 170
Examination of 5 weight loss drugs in biological samples by UPLC-MS/MS.
Aihua Wang
Aihua Wang, Xinxin Ren, Ruihua Wang, Zhongshan Yu
Institute of Forensic Science, Ministry of Public Security
Abstract ID 198
The use of N-Acetyltaurine in urine as a marker for the detection of alcohol consumption in patients pre- and post-liver transplantation.
Hilke Andresen-Streichert
Hilke Andresen-Streichert1, Yannik Beres2, Alexander Müller3, Wolfgang Weinmann4, Martina Sterneck2, Gisela Skopp5
1Institute of Legal Medicine, Department of Toxicology, University Medical Center Cologne, 2Hepatobiliary Surgery and Transplantation Surgery, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, 3Institute of Legal Medicine, Department of Toxicology and Alcohology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, 4Institute of Forensic Medicine, University of Bern, Bern, Switzerland, 5Forensisch Toxikologisches Centrum München GmbH, Munich, Germany

Abstract ID 200
Norcarfentanil may also come from remifentanil treatment.
Helene Eysseric
Helene Eysseric1, Nathalie Allibe2, Théo Willeman1, Nathalie Fouilhè Sam-lai1, Michel Mallaret1, Jean-François Jourdil1
1CHU de Grenoble, 2Université Grenoble Alpes

Abstract ID 211
Evaluation of cannabinoids analysis in the urine and hair of users taking long-term use of hemp products.
Dongeun Park
Dongeun Park1, Byungseok Cho2, Byungjoo Kim2, Ilung SeoF, Eunmi Kim2, Seungkyung Baeck2
1Daejeon Institute, National Forensic Service, 2National Forensic Service

Abstract ID 214
Eugenol ingestion: A case report.
Mila Lovric
Mila Lovric1, Mila Lovri2, Dunja Rogi2
1University Hospital Centre Zagreb, Department of Laboratory Diagnostics, 2University Hospital Center Zagreb, Department of Laboratory Diagnostics

Abstract ID 215
Ingestion of magic mushrooms: A case report.
Mila Lovrić
Mila Lovri1, Andrijana Š avni ar2, Arnes Rešiši2, Dunja Rogi2
1University Hospital Centre Zagreb, Department of Laboratory Diagnostics, 2University Hospital Center Zagreb, Department of Laboratory Diagnostics, 3Children’s Hospital Zagreb, Division of Pediatric Clinical Pharmacology and Toxicology

Abstract ID 231
Optimisation of a fast and easy quantification method for 54 benzodiazepines & Z-drugs, including 20 designer benzodiazepines, in plasma.
Maarten Degreef
Maarten Degreef1, Alexander L.N. van Nuijs1, Kristof E. Maudens2
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1Institute of Legal Medicine, Saarland University, 2Department of Experimental and Clinical Toxicology, Institute of Experimental and Clinical Pharmacology and Toxicology, Saarland University
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1Department of Forensic Sciences, Division of Laboratory Medicine, Oslo University Hospital, Oslo, 2National Board of Forensic Medicine, Linköping, Sweden

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Ersin Göl
Ersin Göl1, Ismet Cok2, Ruchan Ozturk1
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1National Forensic Service, 2Seoul Institute, National Forensic Service

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1UCT Prague, 2Institute Of Criminalistics Prague

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Jadwiga Musiał
Jadwiga Musiał¹, Jakub Czarny², Natalia Galant², Michał Raczkowski², Barbara Przyjazna², Jolanta Powierska-Czarny³, Renata Herman², Magdalena Chrostowska², Paulina Jerszy ska²
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Natalia Galant¹, Jakub Czarny¹, Jadwiga Musia³, Renata Herman¹, Paulina Jerszy ska¹, Jolanta Powierska-Czarny², Michał Raczkowski¹, Barbara Przyjazna¹, Magdalena Chrostowska¹
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Marie Nielsen
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Section of Forensic Chemistry, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen

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Qian Zheng¹, Juan Jia², Tao Wang², Zhiwen Wei², Keming Yun²
¹Shanxi Medical University, ²Shanxi Medical University: Key Laboratory of Forensic Toxicology, Ministry of Public Sencurity, People’s Republic of China
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Eunyoung Park

Eunyoung Park¹, Yongho Shin¹, Junghak Lee¹, Jae Chul Cheong², Hye Suk Lee³, Jeong-Han Kim¹

¹Department of Agricultural Biotechnology, Seoul National University, ²Forensic Chemistry Laboratory, Forensic Genetic & Chemistry Division, Supreme Prosecutors’ Office, ³College of Pharmacy, The Catholic University of Korea

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Mohammad Alses

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University of Damascus / Faculty of Pharmacy

One-step extraction and detection of 513 drugs from hair by LC-MS/MS.

Natalia Galant

Natalia Galant¹, Jakub Czarny¹, Michal Raczkowski¹, Jadwiga Musial², Magdalena Chrostowska¹, Renata Herman¹, Jolanta Powierska-Czarny³, Paulina Jerszy ska¹, Barbara Przyjazna¹

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Drug free campus policy in Ege University, Turkey: The importance of drug monitoring stage.

Ezgi Emen

Ezgi Emen, Duygu Yeşim Karabulut, Serap Annette Akgür

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Abstract ID 54
Oral fluid as an alternative matrix for therapeutic drug monitoring of immunosuppressants.

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Aim
Immunosuppressants constitute the main treatment after organ transplants to avoid rejection, and they are also used in many autoimmune diseases. Their narrow therapeutic index frequently requires blood sampling and monitoring. The main disadvantage of blood is its invasive collection, which could be overcome using oral fluid (OF) for therapeutic drug monitoring (TDM). In addition, OF drug concentration represents the unbound fraction, which may correlate better with the pharmacological and adverse toxic effects. The aims of this study were to develop and validate a method for the determination of 5 immunosuppressants [mycophenolic acid (MPA), tacrolimus (TAC), sirolimus (SIR), everolimus (EVE) and cyclosporin A (CsA)] in OF by LC-MS/MS, as an essential tool to study the usefulness of OF as an alternative matrix for immunosuppressants’ TDM; and to find the best OF collector for these analytes.

Methods
OF samples (0.5 mL) were sonicated 30 s and the internal standard solution was added. Then, samples were submitted to protein precipitation with cold acetonitrile and, after centrifugation, supernatants were evaporated. Following reconstitution in a mixture of methanol:formic acid 5% in water (1:10, v:v), samples were extracted by solid-phase extraction with OASIS HLB cartridges. Chromatographic separation was performed using a guard column connected to an XBridge Shield RP18 column (2.1x100 mm, 3.5 µm), and 2 mM ammonium formate with 0.1% formic acid in water and acetonitrile as mobile phase. Detection was performed in electrospray in positive mode, and two MRM transitions were selected for each analyte with the highest concentrations in OF, followed by MPA (n= 10), >LOD to 1.8 ng/mL for TAC (n= 15), 1.8 to 10.6 ng/mL for CsA (n= 2) and 4.9 ng/mL for EVE (n= 1).

Results
Chromatographic elution of all the analytes was achieved in 5 minutes, with a total run time of 8 minutes. Among the different collection devices evaluated, the maximum recovery was obtained using silanized test tubes (73.5%-107.7%), and the worst results were observed for Salivette® (<35.5%, except for MPA). All validation parameters fulfilled the acceptance criteria: linearity (5-500 ng/mL for MPA, 1-100 ng/mL for CsA, and 0.1-5 ng/mL for TAC, SIR and EVE), accuracy (84.5% to 96.4% of target concentration), imprecision (1.0% to 9.4%), limits of detection (from 0.05 to 1 ng/mL) and quantification (from 0.1 to 5 ng/mL), selectivity (no interferences), matrix effect (-42.7% to -80.5%, %CV= 4.0-10.5%), extraction efficiency (80.7% to 98.8%), stability after 72 h in the autosampler (<13.3% loss), freeze/thaw stability (<18.4% loss) and dilution integrity [93.9%-98.7% of nominal concentrations, except for CsA (75.6%)]. Real OF samples were collected 3-6.5 h after last dose administration by passive drool using silanized test tubes, with doses ranging from 360 to 500 mg for MPA, 25 to 100 mg for CsA, 0.50 to 12 mg for TAC and 0.75 mg for EVE. All the prescribed immunosuppressants were detected in OF, except TAC in one case. Concentrations ranged from >LOD to 684.9 ng/mL for MPA (n= 10), >LOD to 1.8 ng/mL for TAC (n= 15), 1.8 to 10.6 ng/mL for CsA (n= 2) and 4.9 ng/mL for EVE (n= 1).

Discussion
All the previously published methods for immunosuppressants in OF were developed for the determination of just one drug. The present method allows the simultaneous quantification of the 5 immunosuppressants most commonly used worldwide with appropriate sensitivity and using the same OF aliquot (0.5 mL). As these drugs are frequently administered in combined therapy, the method will be very useful for the evaluation of OF samples for TDM of immunosuppressive drugs as an alternative to blood samples. MPA was the analyte with the highest concentrations in OF, followed by CsA, as expected due to their higher therapeutic doses. Although low OF concentrations for TAC and EVE were expected given the low blood reference concentrations and the high percentage of protein binding, both analytes were detected in those patients under TAC or EVE treatment. The only exception where TAC was not detected, was the case of a patient taking a very low dose of this drug (0.50 mg /48 h). Unfortunately, OF specimens from patients taking SIR were not available for analysis.

Conclusions
A LC-MS/MS method for the simultaneous determination of the 5 most common used immunosuppressants in OF was developed and fully validated and satisfactorily applied to real samples. Therefore, this method using silanized test tubes for sampling collection is suitable to explore our next goals: to study possible correlations between blood and OF immunosuppressants concentrations, and to explore the usefulness of OF as an alternative to whole blood for immunosuppressants’ TDM.
Aim
Metabolomics is a powerful emerging approach increasingly used as a characteristic fingerprint in clinical sciences. It has been widely applied in the toxicological field particularly to elucidate the mechanism of toxicity actions, adverse effects and variability of drug responses. Metabolomics entered the clinical toxicology field over a decade ago, showing rapid growth in the comprehensive analysis of plasma, urine and tissue extracts. This study aims to assess the method for the screening of a wide range of endogenous metabolites to assist in the search for cerebrospinal fluid (CSF) metabolic signature(s) in clinical trials.

Methods
Untargeted metabolomics is a qualitative hypothesis generating comparative analysis of changes in detectable metabolites between different phenotypes in a biological system. The screening of a wide range of metabolites were measured using the developed UPLC-HRMS method. A simple methanol pre-treatment protocol were performed on cerebrospinal fluid samples with a mixed internal standard solution was optimised for specimens obtained from the Children's Hospital at Westmead. The chromatographic separation was achieved on the Acquity UPLC HSS T3 column using a gradient elution consisting of 0.1% formic acid and acetonitrile with 0.1% formic acid was used for the separation of metabolites within a 22 minute time frame. The HRMS was operated in the full MS scan mode for m/z 50 to 500 using positive electrospray ionisation. Quality control (QC) samples were analysed to assess the reproducibility of the method. For this study, cerebrospinal fluid samples were spiked with three different concentrations of the mixed standard solution and the QC sample was composed of equal amounts from the three spiked groups. The clustering of the QC samples were assessed using principal component analysis (PCA) according to the normalised peak area data with internal standards.

Results
The untargeted profiling detected over 200 metabolites in CSF. A total of 159 compounds were identified by reference standards and the human metabolome database. This involved the identification of 40 endogenous metabolites including major neurotransmitters such as dopamine, serotonin, histamine, glutamic acid, tryptophan and their metabolites. The PCA score plot showed 95% confidence level and distinct clustering of the QC samples against other spiked groups. The application of untargeted metabolomics requires rapid and reproducible sample preparation methods and the QC results indicate the robustness of the developed analysis method.

Discussion
The CSF matrix contains rich biochemical information which directly interacts with the central nervous system and its close proximity to the disease origin will provide an enhanced representation of neurochemical states. The main limitations is the invasive nature of the matrix and challenges of obtaining samples. For these reasons this work attempts to address the gap in literature. The developed analysis method is in readiness to be applied on a clinical trial provided by the Children's Hospital at Westmead to investigate the small metabolic changes in the central nervous system for diagnosis of specific diseases in its early stages. The developed method simultaneously detected a wide range of neurotransmitters and their metabolites from different metabolic pathways. Several neurotransmitters identified in this method have been found to be involved in a wide of studies in the toxicological field and central nervous disorders.

Conclusions
An untargeted metabolomics analysis method was developed using UPLC-HRMS for the simultaneous identification of forty endogenous compounds in several metabolic pathways. The simple, rapid and reproducible CSF sample preparation procedure together with a short chromatographic run time it will offer an advantage amongst existing protocols. The diagnostic potential of the developed approach warrants further validation by applying it on a clinical trial.
Abstract ID 132
Tobacco markers in meconium, maternal interview and neonatal outcomes.
Ana de Castro
Angela López-Rabuñal, Ana de Castro, Elena Lendoiro, Marta Concheiro-Guisán, Manuel López-Rivadulla, Angelines Cruz
1University of Santiago de Compostela, 2John Jay College of Criminal Justice

Aim
In Spain, about 11.9-25% pregnant women continue smoking during pregnancy, which can cause multiple short and long-term negative effects, such as serious obstetric problems and interference with fetal development. Drug use during pregnancy, including tobacco, is frequently documented through maternal interview; however, recall issues, among others, may affect the accuracy of this report. Meconium is currently considered the reference matrix for the objective identification of drug use during pregnancy, since its analysis allows the detection of direct fetal exposure to drugs, providing a wide detection window (mainly from the third trimester of pregnancy). The goal of this work was to study the possible correlation between analytical results in meconium, self-report maternal tobacco use and neonatal outcomes.

Methods
Participants were pregnant women who delivered at the University Hospitals of Santiago de Compostela and Vigo (Galicia, Spain) from January 2012 to December 2015. After delivery, women were interviewed by the physician about their socio-demographic characteristics (age, education level, work and marital status) and drug use during pregnancy. Data collected from the newborns included Apgar scores at 1 and 5 minutes, weight, length, head circumference and gestational age at birth; and weight, length, head circumference and body mass index (BMI) at 6, 12 and 24 months after delivery. Meconium specimens (n=565) were analysed for nicotine, cotinine and hydroxycotinine by LC-MS/MS, and meconium results were compared with maternal socio-demographic and smoking habits during pregnancy and with neonatal outcomes. Preterm newborns were excluded, and data were stratified by sex. This study was approved by the Ethics Committee of the University of Santiago de Compostela (Spain) and by the Galician Clinical Research Ethics Committee (Xunta de Galicia, Spain).

Results
Median maternal age (n=565) was 33 years old, and ranged from 16 to 46 years old. Smoking habits were significantly and inversely correlated with maternal age and education level (p<0.001), and were more common in unemployed mothers (p=0.001) and those living with a smoker partner (p<0.001). Out of the 565 meconium specimens, 100 were positive for at least one tobacco biomarker. Concentrations ranged from 10-239.6 ng/g for nicotine, 5.8-285 ng/g for cotinine and 2-160.5 ng/g for hydroxycotinine. There were 17.7% positive meconium specimens vs 13.5% mothers admitting tobacco use during pregnancy, and meconium results and interview answers were statistically correlated (p<0.001). Moreover, only 14 mothers whose neonate’s meconium was positive denied tobacco use during pregnancy, which may represent second-hand exposure. Birth weight was significantly lower for newborns with meconium positive results in males (p=0.023) and females (p=0.001), while for length significance was only observed in females (p=0.001); however, when excluding meconium specimens positive for other drugs (n=59; 10.4%), a statistically significant difference was only found for female weight (p=0.045). No significant correlation was found for the remaining neonatal outcomes.

Discussion
We describe the analysis of the largest number of meconium specimens (>500 specimens) to date for which corresponding maternal interview answers containing demographic data and drug habits, and neonatal outcome data were available. Our results show that tobacco biomarkers identification in meconium is more effective than maternal interview for detecting fetal tobacco exposure; nevertheless, a strong positive correlation was found between both parameters (p<0.001), which agree with results from previous studies. As anthropometric parameters were significantly different depending on gestational age and sex, preterm newborns were excluded, and comparisons were done separately for males and females. Neonatal outcome measures were always lower for the group exposed to tobacco than for the non-exposed group, but significant differences were only observed for female neonatal weight.

Conclusions
All these information allowed us to assess the usefulness of meconium analysis compared to maternal interview to detect in-utero tobacco exposure, and to evaluate possible associations between meconium results and neonatal outcomes. This work is the most comprehensive study with similar characteristics and, therefore, very reliable conclusions could be derived from it.
Abstract ID 162
Suspicion of GHB poisoning in a young child highlighted with a rapid enzymatic assay.

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Aim
To respond to the renewed interest in consumption of GHB/GBL in festive usage at the beginning of 2018 in Paris, the laboratory of toxicology of Lariboisière Hospital have changed GHB detection and quantification strategy for rapid response 24/24 to management conditions for intoxicated patients. We described a new case of a metabolic disease in a child from a family with context of addiction, diagnosed rapidly by a GHB dosage. A very sleepy and difficult to wake up 13-month-old child is hospitalized on alert from a physician present in a private party. Cannabis detection in urine with immunological test first base on toxicological diagnosis.

Methods
A further routine toxicological screening was investigated in Lariboisière Hospital with immunological tests (Abbott), confirmation methods in LCMS/DAD and GCMS and a new GHB enzymatic assay (Bühlmann Laboratories AG, Schönenbuch, Suisse) [1]. This assay for GHB was recently adapted in plasma and urine on an Architect® analyzer (Abbott) for routine use 24/24. For each determination, ethanol was estimated in plasma or urine to evaluate known ethanol interference. In this case report, toxicological monitoring of GHB was performed on urine and plasma during hospitalization with this enzymatic test. All the GHB concentrations results were confirmed in GCMS.

Results
On the first urinary sample (day 1), positive THC COOH determined with an immunological test was confirmed by identification in GCMS. THC and 11OH-THC were not detected. Urine GHB concentration with enzymatic assay was observed at 1714 mg/L. Alcohol determination in urine with enzymatic assay was negative. On urine (and plasma) samples at day 4 and day 17, GHB concentrations were 165 mg/L (147 mg/L) and 110 mg/L (93 mg/L). Additional investigations and a complete biochemical diagnose with urinary organic acids in chromatography was carried out : elevated aciduria with 4-hydroxybutyrate was observed.

Discussion
First results in urine with elevated GHB concentration and THC COOH detection with context of mother addiction lead to first conclusions with a toxicological origin of the clinical symptoms. Chromatography of urinary organic acids and elevated concentrations of GHB in urine and plasma quickly oriented the diagnosis towards a metabolic disease.

The young child is identified with a neurometabolic disorder of gamma aminobutyrate (GABA) named Succinic SemiAldehyde DesHydrogenase Deficiency or SSADHD. This disease is a rare genetic disorder described to increase physiological concentrations of GHB by 10 to 20 times. The phenotype encompasses nonspecific neurological features, including developmental delay, hypotonia and neuropsychiatric disturbances and the SSADHD is underdiagnosed. For the young child, several months later, the SSADHD was confirmed by genetic test (mutation of ALDHSA1 gene).

In the case report, presence of THC COOH in the first urinary sample is certainly related to breastfeeding by a cannabis consuming mother. Indeed, after these first positive results in the young child urine, mother admitted her own consumption of THC. The activation of mTOR signalling by THC could perhaps explain the first elevated concentration of GHB in urine [2].

Conclusions
This case report highlights a new interest for this rapid and high throughput assay for detection and quantitative analysis of GHB in biofluids : it might be beneficial for rapid screening for SSADHD in targeted populations [3].

Références
Abstract ID 179
Differences in metabolism of ATM4 between Asian and Caucasian subjects using street heroin.

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Aim
An important toxicology challenge is to evaluate if illicit heroin has been used when the defence is that the urinary morphine has originated from poppy seed ingestion or herbal medicines. In the absence of 6-MAM, the glucuronide conjugate of ATM4 has been recommended as a urinary marker of ‘street’ heroin administration [1]. Consideration of recognised gene polymorphisms between races may result in differences in conversion to, and metabolism of, ATM4 between races. Moreover, street heroin originated from Southeast Asia is highly purified into a white fluffy powder in the 80% to 95% purity range, which is so called ‘Number 4’. It contains less impurities and consequently may greatly limit the detection of putative markers. Our goal was to understand the metabolic profiling of ATM4, and identify specific urinary metabolites for different populations after street heroin administration to comprehend if they are universal markers suitable for global cases.

Methods
The glucuronide and sulfate of ATM4, designated ATM4G and ATM4S, were generated in vitro using human liver microsomes and cytosolic fractions respectively. 800 L of urine was precipitated using 1 mL acetonitrile and centrifuged at 20000 g for 12 minutes at 4 °C. The supernatant was evaporated to dryness under nitrogen and reconstituted with 100 μL 5% methanol in water containing internal standard at 50 ng mL−1. MS3 and neutral loss scan were newly developed to detect trace metabolites in urine in negative mode. ATM4S was analysed by neutral loss with m/z 80 and regular MRM transitions m/z 432 → 352 and m/z 432 → 307. MS3 transitions of ATM4S m/z 432 → 352 → 322 and m/z 432 → 352 → 307 were further used to confirm the presence of ATM4S. In Taiwan, urine samples were collected from patients undergoing a methadone-maintenance treatment program (n=89), from opiate drug abusers (n=463) and from the herbal medicine volunteers (n=20). In the UK, urine specimens were obtained from street heroin abusers (n=50) and volunteers having ingested 6 g of poppy seeds (n=6).

Results
ATM4S, a new sulfated metabolite of ATM4, was identified in 26 of 552 samples in Taiwan. Approximately 73% (19 out of 26 cases) of positive samples confirmed by MS3 showed co-eluting interferences that resulted in the signal-to-noise (S/N) lower than 3 and 1 if using MRM scan. In ATM4S positive samples, 7 specimens can be identified by MRM and only 2 were detected by neutral loss scan showing S/N >7. Qualification using MS3 eliminated uncharacterised interferences greatly and disclosed the trace amount of ATM4S which was imbedded in complex background. By contrast, ATM4G which is a glucuronide conjugate of ATM4 was found in 27 of 50 samples from the UK with averagely S/N =7. No ATM4, ATM4G or ATM4S was detected in urine collected from users of herbal medicine or following poppy seed ingestion.

Discussion
Street heroin synthesized in Southeast Asia may be extracted and considerably purified (‘white heroin’), resulting in relatively smaller amounts of trace impurities compared to elsewhere. Therefore, a sensitive MS3 method was required to detect urinary ATM4S at trace level. The current method sensitivity was also limited by the chemical nature of ATM4S because negative mode scan is necessary of which the sensitivity is approximately one tenth of positive mode here. All factors may account in the low detection rate and low concentration of ATM4S in Taiwanese samples. Notwithstanding, ATM4S was only found in Southeast Asian heroin abusers’ urine while ATM4G was only detected in Caucasian specimens. This is likely to be due to genetic polymorphisms in phase 2 enzymes between the two populations.

Conclusions
ATM4 is subject to different metabolic pathways between populations, notably phase 2 metabolism. Comprehending the complete metabolic profiling of putative markers and select proper specific urinary metabolites for different populations are essential to be considered by toxicology laboratory. Detection of its sulfated metabolite (ATM4S) and glucuronide conjugate (ATM4G) together can offer a greater degree of retrospection of drug detection for different races compared with that of 6-MAM.

Reference:
Abstract ID  194
A Simple Online Extraction LC/LC Atmospheric Pressure Ionization (APCI) MS/MS Assay for the Analysis of 17 Cannabinoids and Metabolites in Human Plasma.

Cristina Sempio

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Aim
In recent years, Cannabidiol (CBD) has been studied for a variety of therapeutic applications alone or in combination with Δ9-tetrahydrocannabinol (THC) but little is known about its pharmacokinetics and metabolism. Cannabis consumption is generally monitored by the detection of THC and its metabolites, 11-hydroxy-THC (11-OH-THC) and 11-nor-9-carboxy-THC (THCCOOH) and in some cases CBD, in plasma or urine. Recently, the surge of preclinical, clinical and observational trials involving THC and CBD increased the need for sensitive and specific analytical assays to establish dose-effect relationships. We developed and validated an online extraction high-performance liquid chromatography coupled to tandem mass spectrometry (LC/LC-MS/MS) method for simultaneous quantification of 17 cannabinoids and metabolites including THC, 11-OH-THC, THCCOOH, THCCOOC glucuronide (THCCOOH-gluc), THC glucuronide (THC-gluc), CBD, 6α-hydroxy-CBD (6α-OH-CBD), 6β-hydroxy-CBD (6 β-OH-CBD), 7-hydroxy-CBD (7-OH-CBD), (3S-trans)-CBD-7-oic Acid (7-CBD-COOH), CBD glucuronide (CBD-gluc), cannabiol (CBN),cannabigerol (CBG), cannabidivarin (CBDV), cannabichromene (CBC), Δ9-tetrahydrocannabinol (THCV) and 11-nor-9-carboxy-THCV (THCV-COOH) in human plasma. The novelty of this approach is the inclusion of the CBD metabolites which were currently made commercially available or were produced in-house (e.g. CBD-gluc).

Methods
CBD-gluc standard is not commercially available so it was produced in-house by isolation of CBD-gluc from urine of patients enrolled in an IRB-approved clinical study using pure CBD oil. Chemical structure confirmation and quantification were performed by 1H-NMR and fragmentation mass spectrometry. For calibration standards and quality control samples, human plasma was spiked with cannabinoids at varying concentrations within the working range of the respective compound and 200 µL was extracted using a simple one-step procedure. Eight hundred µL of protein precipitation solution (methanol/0.2M ZnSO4, 7:3, v/v) containing the internal standard mix at a concentration of 5 ng/mL was added. Samples were vortexed for 10 min and centrifuged (4°C, 26,000g, 10 min). After centrifugation, supernatants were transferred into HPLC vials. The extracts were analyzed using online trapping LC/LC-APCI-MS/MS. Two-hundred and fifty µL of the sample supernatant was injected onto the extraction column (3.0x5.0 mm, 2.7 µm particle size, C8) and was washed with a gradient flow of 0.5-1.5 mL/min over 1 minute. The analytes were back-flushed onto the analytical column (3.0x150 mm, 2.7 µm particle size, RP-Amide). Mobile phases were 0.1% formic acid and a mixture of acetonitrile/methanol/isopropanol (60:20:20). The HPLC system was interfaced with an API5000 MS/MS via an atmospheric pressure chemical ionization source (APCI). The mass spectrometer was run in the positive multiple reaction monitoring (MRM) mode.

Results
During method development, various chromatographic conditions and chromatography columns have been tested. However, all conventional phenyl, perfluorophenyl, C8 and C18 reverse phase columns lacked the ability to separate 7-OH-CBD from 6β-OH-CBD. The use of the RP-Amide column with its strong H-bonding properties between the amide carbonyl (H-bond acceptor) and H-bond donors (hydroxy groups) resulted in a good separation of these structural isomers. The method was validated according to FDA guidelines; matrix effect and recovery were evaluated according to Matuszewski et al. in 6 individual lots. Linear ranges were 0.39-400 ng/mL for THC, THCCOOH, CBD and CBDV; 1.56-400 ng/mL for 11-OH-THC and 6α-OH-CBD; 3.13-400 ng/mL for 7-OH-CBD; 7.8-2000 ng/mL for THCCOOH-gluc; 0.78-100 ng/mL for CBD-gluc; 0.78-200 ng/mL for THC-gluc and 0.78-400 ng/mL for all the other compounds. Acceptance criteria for intra- and inter-batch accuracy (85-115%) and precision (<15%) were met for all compounds in plasma. Mean extraction efficiency ranged 48.5-101% in plasma. Mean absolute matrix effect ranged 6.08-68.0% in plasma, except for CBD-Gluc (169%). No carry over was detected. To date, 219 plasma samples collected during clinical and observational studies in marijuana product users were analyzed with the presented assay. One-hundred and seventy-five samples were positive for CBD, 201 for 7-CBD-COOH, 168 for CBD-gluc, 122 for 7-OH-CBD, 76 for 6α-OH-CBD and only 2 for 6β-OH-CBD.

Discussion
This assay is the first validated assay that includes all major CBD metabolites in addition to THC and its major metabolites and other minor cannabinoids. Especially for larger observational clinical studies, in which investigators have less control over the administered dose and time of administration, it is essential to monitor CBD and its metabolites to be able to better estimate CBD exposure.

Conclusions
We present a validated, high-throughput, sensitive and specific assay for the quantification of seventeen cannabinoids and key metabolites that can be used for clinical monitoring and research studies.
Abstract ID 236

Analysis of Mushroom and Plant Toxins in Human Urine by Means of Normal Phase Liquid Chromatography Coupled to High Resolution Mass Spectrometry.

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Aim

The identification of mushroom and plant toxins at an early stage of a suspected poisoning can be crucial for therapeutic decision making. Therefore, the aim of this study was to develop a liquid chromatography (LC)-high resolution mass spectrometry (HRMS) method for identification of mushroom and plant toxins such as alpha- and beta-amanitin, psilocin, bufotenine, muscarine, muscimol, ibotenic acid and ricinine in human urine.

Methods

Neat urine samples were centrifuged at -10°C, 15000 rpm for 10 minutes. The supernatant was used for a reversed-phase liquid-liquid extraction (rp-LLE) with dichloromethane. One part of the aqueous phase was used for solid phase extraction (SPE, Strata-X-Drug-B columns, 60 mg/3 mL; Phenomenex, Aschaffenburg, Germany) and one part for urine precipitation (UP) by acetonitrile. After combining SPE and UP, the toxins were chromatographically separated using a normal phase column (HILIC NUCLEODUR 100/2/1.8, Macherey-Nagel, Düren, Germany). The used LC system (Dionex UltiMate 3000 RS) was coupled to an Orbitrap-based mass spectrometer (Q-Exactive Focus, Thermo Fisher Scientific, San Jose, USA). Acetonitrile and methanol were used as main eluents for HILIC separation. The method was validated as recommended for qualitative procedures including extraction recoveries, analytical selectivity, and applicability.

Results

Due to the high structural diversity of the compounds, only alpha- and beta-amanitin, psilocin, bufotenine, and ricinine could be extracted by SPE but adding the UP supernatant allowed the recovery of all analytes. LODs were 1 ng/mL for alpha- and beta-amanitin, 5 ng/mL for muscarine, psilocin, bufotenine, and ricinine, and 1.5 mg/L and 2 mg/L for ibotenic acid and muscimol, respectively. Recovery ranged from 80% - 100% for alpha-amanitin, beta-amanitin and muscarine and was 75% for bufotenine, 68% for muscimol, and 48% for ibotenic acid. Rather low recoveries were observed for psilocin and ricinine (both 35%). Gamma-amanitin, L-tryptophan-d5, and psilocin-d10 were shown to be appropriate internal standards, which were able to compensate recovery variations and matrix effects, except for bufotenine and ricinine. After selectivity was demonstrated by analysing 10 urine samples from different individuals, the method was finally applied to urine samples from intoxicated patients after suspected consumption of mushrooms. In two samples, both alpha- and beta-amanitin could be identified, in two additional samples only alpha-amanitin. Muscimol, ibotenic acid, and muscarine could be identified in a urine sample of a patient after ingestion of Amanita muscaria or Amanita pantherina. In two other urine samples, psilocin-O-glucuronide could be found in addition to psilocin. Furthermore, the results for alpha- and beta-amanitin were confirmed using an established LC-HRMS/MS reference method.

Discussion

Sufficient separation of analytes could only be achieved by HILIC. Particularly the separation of alpha- and beta-amanitin, bufotenine and psilocin as well as muscimol and ibotenic acid was critical. The mass spectral information together with the retention times were shown to be selective and sensitive enough for analyte identification. The low recovery of psilocin could at least in part be explained by decent degradation during the extraction procedures. The internal standard psilocin-d10 compensated for the low recovery and particularly the matrix effects. Since psilocin-O-glucuronide is excreted in higher amounts and is known to be more stable, it is a more suitable target for urine screenings. Unfortunately, validation experiments could not be performed as it is commercial not available.

Conclusions

We successfully developed an analytical approach for analysing several toxins to support therapeutic decision making in a clinical toxicology setting. After development, the method was applied to detect toxins in human urine samples after suspected ingestion of mushrooms. To our knowledge, the presented method is the most comprehensive approach for identification of mushroom toxins in a human matrix so far.
Abstract ID 263

All or Nothing Retrospective Evaluation of EtG Adherence Analysis Results in the Context of Organ Transplantation.

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Aim
According to the Guidelines of the German Medical Association, patients with alcohol-induced liver cirrhosis are only included in the waiting list for liver transplants after at least six months of alcohol abstinence. In this context, ethyl glucuronide (EtG) testing is performed in urine using a 0.5 µg/mL cut-off. Positive immunoassay testing results have to be confirmed by LC-MS/MS. While on the list, patients have to be tested for urinary EtG at least every three months. In case of evidence for continued alcohol consumption they are considered “not transplantable” and a new evaluation is started. The aim of the presented study was the retrospective evaluation of EtG analysis data obtained from 2017 to March 2019.

Methods
Methods: Urine specimens were first tested for EtG using the EtG DRI kit (Thermo Fisher Scientific, Dreieich, Germany) according to manufacturer’s specifications. Suspicious (cDRI > 0.25 µg/mL), positive (cDRI > 0.5 µg/mL) or highly positive (cDRI≥2.0 µg/mL) urine specimens were submitted to confirmation analysis using a fully validated LC-MS/MS method using a HILIC based chromatographic system (EC 150 Nucleodur HILIC, 100 x 4.6 mm, 5 µm; Macherey-Nagel, Düren, Germany) and a QTrap 4000 instrument (AbSciex, Darmstadt, Germany) operated in MRM mode. Additionally, urinary creatinine concentrations were determined by the DRI™ Creatinine-Detect™ assay (Thermo Fisher Scientific, Dreieich, Germany). Statistical analysis was performed by GraphPad Prism 5.0 (San Diego, USA).

Results
Results and Discussion: Approximately 70 % (2,805 of 3,972) of the specimens to be tested for EtG specimens were from male patients.

For 60 of 3,972 samples to be tested for EtG, analysis by DRI failed due to sample properties (e. g. turbidity). For these samples, EtG analysis was performed by LC-MS/MS only.

464 (~11%) of 3,972 urine specimens were classified as positive according EtG DRI kit (cDRI > 0.5 µg/mL). Within that sample set about 66 % of the samples (n= 312 of 464) had urinary EtG concentrations higher than the DRI calibration range (cDRI≥ 2.0 µg/mL). All of these samples were confirmed positive by LC-MS/MS. In most of those cases urinary ETG concentrations were also above the calibration range of the LC-MS/MS approach (cLC-MS/MS > 9.0 µg/mL).

Significant differences (Kruskal-Walis test; p < 0.0001) of mean/median creatinine levels could be found for negative (cDRI= 945 µg/mL / 770 µg/mL; n= 3,314), suspicious (cDRI= 1,495 µg/mL / 1,281 µg/mL; n= 135), positive (cDRI= 1,170 µg/mL / 1,010 µg/mL; n= 152) and highly positive (cDRI= 1,342 µg/mL / 1,257 µg/mL; n= 312) samples, while creatinine concentrations of over 11,000 clinical samples (sent to the author’s lab) were in accordance with literature data showing a left skewed frequency distribution with a mean/median of cDRI= 1,220 µg/mL / 1,036 µg/mL (n= 11,094). In accordance with literature data no gender effect was observed for the positive (n= 152) and highly positive (n= 312) samples.

Systematic evaluation of the DRI cut-off was performed on a subset of samples (2017-2019 n= 2,827) using suspicious (cDRI > 0.25 < 0.5 µg/mL; n= 107) and positive samples (cDRI≥0.5 < 2.0 µg/mL; n= 111). Bland-Altman analysis for DRI and LC-MS/MS data revealed higher DRI values in comparison to LC-MS/MS with a mean systematic offset of 0.186 µg/mL. This offset was found to be independent of the DRI reagent batches or the determined EtG concentrations. For 5 samples (~5%) of 111 positive (cDRI≥0.5 < 2.0 µg/mL) samples no EtG was detected by LC-MS/MS analysis. In 32 of 111 cases (about 29%) the presence of EtG was confirmed by LC-MS/MS but the cLC-MS/MS was found be below the cut-off of cLC-MS/MS= 0.5 µg/mL.

Discussion
see above

Conclusions
Conclusion: Following a kind of “all or nothing principle”, it was shown, that only in a small percentage of the investigated samples, EtG was present with a cLC-MS/MS >0.5 µg/mL. However within that group, about 66% of the samples contained very high EtG concentrations with cLC-MS/MS >9.0 µg/mL. The recent analysis strategy using primary classification by DRI (DRI cut off cDRI> 0.5 µg/mL) and confirmation and quantification by LC-MS/MS was found to be effective as the data indicated a low risk of false negative classification result. The total false positive rate was low (~5%) with respect to the presence of EtG, but fairly high (~29%) with respect to EtGcLC-MS/MS >0.5 µg/mL.
Comparing the Diagnostic Value of PEth and CDT in 6705 patients: relation to Age and Sex.

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Aim
The direct alcohol biomarker phosphatidylethanol (PEth) has been widely used over the latest years. There is however few large studies comparing the analyses of PEth and Carbohydrate-Deficient Transferrin (CDT) in real life clinical materials. The aims of this project were to compare the diagnostic value of PEth and CDT as biomarkers of alcohol consumption in a large clinical sample and to evaluate the concentration levels in relation to age and sex.

Methods
PEth and CDT concentrations from a clinical chemistry database were included, together with information of age and sex. Samples were mostly collected from primary care physicians in addition to some from social care institutions. Only samples where CDT and PEth were measured simultaneously were used, and only one measurement per patient was included. PEth-concentrations were determined using a Waters Acquity UPC2 (TM) Ultra Performance Convergence chromatography system connected to Waters TQ-S triple quadrupole mass-spectrometer. CDT was quantified by electrophoretic separation of human transferrin using a CapillarysTM System. Values ≥1.7% for CDT and ≥0.31 µmol/L for PEth were considered to represent harmful alcohol consumption. For PEth, higher concentration intervals were also studied, according to previous literature.

Results
6705 patients with values of PEth and CDT measured simultaneously were included. The median age was 54.5 years, with 66 % males and 34 % females. Of the patients showing PEth concentrations higher than 0.30 µmol/L (n=3208), 1701 patients had a CDT value below 1.7 % and 1507 patients had a CDT value at 1.7 % or above. Of the patients showing CDT concentrations at 1.7 % or higher (n=1675), 168 patients had a PEth value below 0.30 µmol/L and 1507 patients had a PEth value above 0.30 µmol/L. Cohen’s kappa for inter-rater reliability between the two methods for diagnosing heavy alcohol use (CDT ≥ 1.7 % or PEth > 0.30 µmol/L) was 0.43 (p < 0.001) overall, 0.45 (p < 0.001) for men and 0.38 (p < 0.001) for women. The kappa values were quite similar across age groups. The number of positive CDT cases increased from 0.6 % in the group with PEth values below 0.03 µmol/L to 78 % in the group with PEth values above 2.5 µmol/L. The overall correlations between age and CDT was significant for men (Spearman’s rho 0.186, p < 0.001), but not for women (Spearman’s rho 0.028, p = 0.183). The correlation between PEth and age was quite similar for men and women (Spearman’s rho 0.110 and 0.097, respectively, p < 0.001). The overall median concentration of CDT in males was significantly higher than in females (p=0.002), but no sex difference was seen for PEth (p=0.553).

Discussion

Conclusions
In this large clinical material, a high number of patients showed PEth values above the suggested cut-off for heavy drinking, but normal CDT values. The present study therefore verifies the superior sensitivity of PEth compared to CDT. The inter-rater reliability between the two biomarkers is surprisingly low, considering that they are interpreted quite equally. The effect of age seems to be minor. The effect of sex seen on CDT, but not on PEth, indicates that PEth, as opposed to CDT, might be formed equally in men and women. Therefore, the issue of gender bias that is possibly present for CDT might be avoided for PEth.
Abstract ID 367
Severe cholinergic syndrome after accidental poisoning with chlormequat solution filled in a soft drink bottle.
Daniela Remane

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Aim
Daniela Remane1, Ingo Hartter2 contributed equally

Introduction: The quaternary ammonium compound chlormequat, also known as chlorocholine-chloride, is used as plant growth regulator. In mammals it has a direct agonistic effect on the muscarinic and nicotinic acetylcholine receptors and is further proposed to inhibit cholinesterase. Common cholinergic symptoms during chlormequat poisoning are bradycardia, cardiac dysrhythmias, ventricular fibrillation with cardiac arrest, hypersalivation, pulmonary edema, increased sweating, confusion, coma, and seizure. Moreover, it causes increased depolarization of the motoric endplate with subsequent muscular impairment and consequently bradypnoea and respiratory arrest. Only few cases of chlormequat poisoning have previously been described, most of them with a fatal outcome. According to our knowledge, this is only the second case of survived chlormequat ingestion with corresponding analytical results regarding chlormequat and its pharmacokinetics.

Case history: A 77-year old male owner of a nursery accidentally swallowed approximately 50 mL of a chlormequat chloride solution (720 g/L), which was filled into a soda-bottle without proper labelling. 10 to 15 minutes after ingestion, the man began to suffer from hypersalivation, sweating (hyperhidrosis) and dizziness. He subsequently collapsed, followed by a trauma on the head. When the ambulance arrived they found an unconscious patient (GCS 3) with a low respiratory rate, bradycardia, hypersalivation, hyperhidrosis and miosis. The patient was intubated and atropine was applied. At the intensive care unit he developed a tachyarrhythmia, hypertension, and hyperglycemia. The patient was unconscious without sedation and mechanically ventilated. A CT-scan of the brain with CT-angiography and blood analysis was performed. Due to the hypersalivation, saliva and clear tracheal mucus had to be removed by suction. To prevent further chlormequat absorption charcoal in combination with a laxative was applied. Initially, a sufficient ventilation of the patient could be achieved under sedation. Hypersalivation and tracheal mucus production was regressive. In consequence, ventilation was deescalated on day 7 and the patient was discharged in a healthy state on day 15. Serum (n=7) and urine (n=3) samples as well as gastric and bottle content were collected for toxicological analysis at different time points.

Methods
Method: Serum samples (100 µL) containing 10 µL internal standard chlormequat-d4 (0.005 µg/µL) were precipitated with 100 µL acetonitrile and 50 µL of the supernatant was dried under nitrogen. After reconstitution in 1000 µL initial mobile phase and samples were analyzed by liquid chromatography-high resolution mass spectrometer using a QExactive Focus instrument coupled with a Dionex UHPLC system (Thermo Fisher Scientific). Gradient elution was performed on a Waters XBridge BEH C8 (2.5 µm; 100 mm x 4.6 mm) using ammonium formate buffer and acetonitrile as mobile phases. The mass spectrometer was operated in the full scan mode with resolution of 17,500 in addition to a PRM MS/MS experiment. Identification and quantification was realized using accurate MS/MS and FS MS with a mass accuracy of at least 5 ppm. The method was validated in serum according the protocol for validation of methods to be used for single case analysis (Peters et al., FSI 2007). Furthermore, urine samples, gastric content and the bottle content were analyzed as described above.

Results
Validation showed good linearity in the calibration range (0.1 mg/L – 15 mg/L) as well as accurate (14.8% QC low, 10.2% QC med, -1.6% QC high) and precise (intraday CV 2.3% QC low, 4.5% QC med, 2.1% QC high) quantification. The limit of quantification was 0.1 mg/L. Chlormequat was stable under bench top conditions. Chlormequat was identified in all serum samples as well as urine, gastric and bottle content. The maximum concentration measured in the first serum sample taken about 45 min after ingestion was 14.2 mg/L. Initially, there was a rapid decrease in concentration but chlormequat was still detectable with 0.15 mg/L about five days after ingestion.

Discussion
Here we describe a case of an unintentional ingestion of approximately 28 mg chlormequat with subsequent severe cholinergic symptoms. In contrast to the inhibitory effect of chlormequat on cholinesterase, as proposed in the literature, serum cholinesterase about two hours after ingestion was in the normal range. The maximum chlormequat concentration in the present case was much higher than that observed after a suicidal injection of chlormequat (Boumrath et al., FSI 2016) and a non-fatal suicide attempt (Yang et al., Forensic Med. Anat. Res. 2015). This might be due to the mode of application, the quick and extensive medical treatment in the present case or due the analytical method used by Boumrath et al., where the chosen calibration range was below the actual measured concentration. The rapid decrease of concentrations within the first 24 hours in the present case is in agreement with the finding of Yang et al. However, chlormequat was still present at 0.15 mg/L in the serum sample collected about 120 hours after the first sample indicating a second slower phase of elimination.

Conclusions
Abstract ID 422
Hide and seek: overcoming the masking effect of opioid antagonists in activity-based screening tests.

Annelies Cannaert
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Aim
Previously we developed an activity-based screening assay for (synthetic) opioids/opiates in biological matrices, allowing the detection of all opiate/opioid substances regardless of their (un)known structure (Cannaert et al., Clin Chem, 2018). This approach had one major limitation: because opioid receptor antagonists (e.g. naloxone) can prevent the in vitro opioid receptor activation induced by opioid agonists, some opioid positive samples were missed. As it is difficult to know if a person with a (potential) opioid overdose received opioid antagonists as treatment, it is important to be able to assess if an antagonist is present in the sample. Therefore, we adapted our protocol to distinguish between 'real negative' samples and 'negative' samples due to the presence of e.g. naloxone.

Methods
In the bioassay, 20 µL of blood extract is applied onto stable cell lines, in which activation of the µ-opioid receptor can be monitored via a split-luciferase system. By adding a small amount of agonist (0.5 ng/mL hydromorphone) after 30 min, we can determine if an opioid antagonist is present in the extract. The applicability was assessed by blind-coded analysis of 136 authentic blood extracts and comparing the results to those of a GC-MS and LC-Q-TOF analysis.

Results
In the bioassay, four distinct profiles can be distinguished: 1) the samples that show net opioid activity (indicating the presence of opioid agonists); 2) samples that do not contain any opioid agonists nor antagonists; 3) samples that contain opioid antagonists at sufficiently high concentrations to block receptor activation; 4) samples that contain a combination of opioid agonists and antagonists. Application on authentic blood samples resulted in a sensitivity and specificity of respectively 98.6% and 95.5%.

Discussion
If the bioluminescent signal rises before the addition of a small amount of agonist, there are opioid agonists present (1 and 4). If no signal is formed before addition, either no agonists are present or there is an antagonist present that blocks the receptor activation (thus light formation). Addition of the small amount of agonist during the assay should normally lead to an increase in signal. If the signal of the extract has the same profile as the blank, no opioids are present (2). If the signal of the extract is lower than the blank, an antagonist is present (3 and 4). Within the 136 blood extracts, there were 42 duplicates, which all gave the same result, showing the robustness of the assay. After subtracting the duplicates, we ended up with 94 unique samples of which 71 of the 72 opioid positive samples (agonist and/or antagonist) could be identified, resulting in a sensitivity of 98.6%. The missed sample contained 11ng/mL of hydrocodone. 21 of the 22 opioid negative samples were correctly scored negative (specificity = 95.5 %). The falsely positive scored sample was suspected to contain a small amount of antagonist, although this was not confirmed.

Conclusions
This improved bioassay allows the simultaneous screening of opioid agonistic and antagonistic activity in biological matrices, thereby further supporting its application as a useful first-line screening tool to investigate potential opioid intoxications in clinical/forensic settings, complementing conventional analytical methods which are currently used.
Abstract ID 467
Monitoring drugs causing serious intoxication in the community through Emergency Department Admission Blood Psychoactive testing-Protocols & initial findings.
Peter Stockham

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Aim
Hospital level data from hospital emergency departments (EDs) regarding drugs involved in intoxication cases holds the potential to provide a very useful source of information relating to harmful community drug use patterns. However, it is often the case that EDs have limited capability for comprehensive toxicological analysis. Data relating to non-fatal drug intoxications at the ED are therefore often based on subjective observations of treating clinicians, which has been shown to be limited in terms of accuracy and reliability.

The aim of the study is to improve knowledge on the drugs associated with ED drug intoxication cases. This was achieved through a collaboration between ED clinicians and forensic toxicologists that enabled comprehensive toxicological analysis on de-identified patient blood samples. Following a successful small scale pilot study (n=103) in 2017-18, and receipt of funding from the National Centre for Clinical Research on Emerging Drugs (Australia), the program was expanded to cover four public hospitals across metropolitan Adelaide, commencing March 2019.

A sharp escalation of sample numbers was expected (600-1000/12 mths), and more efficient protocols for comprehensive targeted screening were required.

A systematic non-targeted ‘suspect screen’ for emerging novel psychoactive substances (NPS) using a crowd sourced database was also investigated to address the problematic detection of these substances.

Methods
Human research ethics approval was obtained, including blood collection with waiver of consent. Patients >18yo, presenting at the ED with symptoms of drug intoxication, and for whom insertion of an intravenous cannula was clinically required, are enrolled. This provides excellent patient coverage as intravenous cannula are used routinely in such cases. A clinical information sheet is used to record data including the setting of drug use, symptomatic and demographic data, and the clinical outcome. Two 5mL blood samples (NaF/oxalate vacuum tubes) are drawn from subjects and stored at -20oC prior to transport to FSSA’s laboratory. The blood samples and clinical data are linked by a unique study number.

Single analyte assays include alcohol (gas chromatography-flame ionisation detector (GC-FID), THC (supported liquid extraction (SLE) – Liquid chromatography mass spectrometry) and γ-hydroxybutyrate (GHB, acidification and liquid-liquid extraction (LLE) - gas chromatography mass spectrometry).

The principal drug screen covers a further 500 drugs, including 200 NPS and uses a recently validated SLE-liquid chromatography-quadrupole time-of-flight mass spectrometer (LC-QTOF) method. A subset of 40 common opioids, antidepressants, benzodiazepines and other common drugs is also analysed quantitatively in the assay. The SLE extraction procedure follows: blood (400µL) is diluted with 0.1% ammonia buffer (400µL) and ethanol (150µL), loaded onto the cartridge (Biotage Isolute, 1mL), eluted with CH2Cl2:iPrOH (5:95, 2.5mL) followed by MBE (2.5mL), evaporated and reconstituted in 50:50 ethanol:0.1% formic acid buffer (100µL). 0.3µL is analysed on the instrument in data-dependent acquisition (DDA) mode for targeted analysis. A further 5µL of the sample extract is analysed on the LC-QTOF using the same LC conditions, but with the QTOF operated in data-independent acquisition mode (DIA) at 3 collision energies. The aim of this is to improve detection of low-dose targeted NPS and to allow non-targeted ‘suspect screening’ for nearly 800 NPS based on accurate parent drug mass and fragment ions (data sourced from HighResNPS.com).

Results
The SLE method described above provides significant time savings compared to the group’s previous LLE screening method through inclusion of additional compounds (e.g. morphine, O-6-acetylmorphine, pregabalin, benzoylcegonine) and faster laboratory processing time. A preliminary process for NPS suspect screening was established using HighResNPS.com database.

Preliminary data shows (n=157 samples) 57% of the patients displayed symptoms consistent with CNS depression, 29% with CNS elevation, and 7% with combined symptoms.

Prevalent drugs included methamphetamine (41% of samples, average 0.27mg/L, median range 0.01-1.4mg/L), alcohol (37%, 0.154%, 0.01-0.323%), diazepam+nordiazepam (23%, combined average 0.50mg/L, 0.04-1.5mg/L), THC (17%, 8.4µg/L, 2-59µg/L) and GHB (15%, 118mg/L, 10-210mg/L). Pregabalin was detected in a significant number of cases (9%). Polydrug use was virtually ubiquitous in the cohort. Targeted NPS detected included etizolam and N-ethylpentylone. 75% of all GHB positive samples also contained methylamphetamine (average 0.27mg/L range 0.01-1.4) with the predominant observed pathology being CNS depression, despite elevated methamphetamine concentrations.

Discussion
The newly developed SLE extraction provides improved scope of analysis with the inclusion of morphine, O-6-acetylmorphine, pregabalin, benzoylcegonine, and significantly faster laboratory processing time. A preliminary process for suspect screening was established using HighResNPS. This approach is not within the normal scope of the vendor software capability; however software and process workarounds were developed to import and screen against the NPS database. Significant findings include the prevalence of methamphetamine, GHB and pregabalin, and the mismatch between observed pathologies and drugs detected.

Conclusions
The assays provide a suitably wide scope of analysis for this project. This collaborative project is providing clinicians, early warning systems and health agencies accurate, analytically confirmed data on local acutely harmful drug use. Work is underway to establish similar ED Clinician/forensic toxicology collaborations nationally.
Abstract ID 473
Alex Lawson
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Aim
Arterial hypertension is one of the most preventable causes of premature morbidity and mortality with resistant hypertension reported to be present in 5 to 30% of the total hypertensive population. Despite the poor prognosis, as many as 53% of those with resistant hypertension are reported to be non-adherent to their prescribed medication. This has led ourselves and others to develop LC-MS/MS methods to screen for the presence of antihypertensive drugs in urine. Despite the success of these assays, many suffer from not offering sufficient analytical breadth to cover all drugs prescribed in hypertension and not being able to identify key metabolites for some classes of drugs. Therefore we have developed a novel method for the detection of 49 commonly prescribed antihypertensive, antidiabetic and cardiovascular disease reducing medications in urine using LC-QTof MS.

Methods
Urine samples from 50 patients attending the hypertension clinic were analysed using an existing LC-MS/MS method and the new LC-QTof MS method. For the LC-QTof MS method, urine samples were prepared using solid phase extraction (Waters HLB Prime) and analysed using a Waters Acquity UPLC system coupled to a Waters Xevo G2-XS QTof MS run in both positive and negative modes using the pre-supplied methods for toxicology screening in the UNIFI program. Chromatographic separation was achieved using gradient elution with a Waters UPLC HSS C18 column, 1.8 µm, 2.1 mm x 150 mm. For positive mode mobile phases were 5 mM ammonium formate pH 3 (A) and acetonitrile containing 0.1% formic acid (B), for negative mode mobile phases were 0.001% formic acid (A) and acetonitrile containing 0.001% formic acid. Source parameters were as follows: capillary voltage 0.80 kV, cone voltage 25 V, cone gas flow 20 L/h, source temperature 150 °C, desolvation temperature 400 °C and desolvation gas flow 800 L/h. Criteria for positive identification of drugs were a retention time (± 0.1 min) when compared to the Waters Toxicology Library and mass error (± 5 ppm) when compared to the assigned molecular formula at low energy. At high energy, presence of at least one accurate fragment ion present in the Waters Toxicology Library is needed for further confirmatory purposes. Intensity of the low energy peak had to be > 1,000 counts. Results obtained, and limit of detection for a number of drugs, were compared between the two methods.

Results
Limit of detection was comparable between the two methods with the majority of drugs detectable at 1 ng/mL using the criteria outlined above. The newer LC-QToF MS method detected all drugs found using the original LC-MS/MS assay, in addition to prescribed drugs not screened for by the existing methodology. Felodipine, lercanidipine and lacidipine could not be identified in the urine of patients who were prescribed these drugs. However, using the available literature on the metabolism of these compounds together with MSE data, we were able to identify metabolites of these drugs (termed felodipine M3, lercanidipine PA3 and lacidipine M3) in patients known to be adherent to these medications.

Discussion
The LC-QToF MS method presented shows similar sensitivity to existing LC-MS/MS assays but is able to screen for more drugs and metabolites and, due to the nature of data acquisition, is flexible should more drugs need to be added to the screen. The results presented here also show that interrogation of MSE datasets is a suitable technique to identify metabolites of drugs of interest should parent compounds not be present in target biological matrices.

Conclusions
Abstract ID 478
New Psychoactive Substances Abuse Situation in Singapore.
HOOI YAN Moy
HOOI YAN Moy1, Yi Ju Yao2, Ching Yee Fong2, Chi Pang Lui2
1HEALTH SCIENCES AUTHORITY,
2Health Sciences Authority

Aim
Abuse of new psychoactive substances (NPS) has been increasing since 2011 in Singapore. Here, the abuse of NPS changed from methylone and 4-methylmethcathinone (or mephedrone) between 2012 and 2014, to ethylone and para-methoxymethamphetamine (PMMA) between 2015 and 2016, and then to MMB-FUBINACA and 5-fluoro-MDMB PINACA between 2017 and 2018. Till date, our laboratory had detected over 60 NPS and their metabolites, mostly synthetic cathinones and synthetic cannabinoids, in suspected NPS abusers' urine specimens that were submitted to our laboratory by enforcement agencies for drug testing. The detection of NPS in urine has been very challenging particularly because of the continuous emergence of newer NPS in the illicit market. In Singapore, the control of NPS is either through generic listing of the structural classes or through listing of the specific NPS. As a result of the continuous emergence, the review of the list of controlled NPS in our legislation needs to be carried out periodically. In this presentation, the approach for legislative control of NPS and the types of NPS/metabolites detected in abusers' urine specimens in the last two years will be discussed.

Methods
The technique used for the identification of NPS in urine is liquid chromatography with high resolution accurate mass hybrid quadrupole-orbitrap mass spectrometer (LC-Orbitrap MS) operated in full scan and data-dependent MS2 acquisition mode with inclusion list covering wide range of target compounds. To build the inclusion list, the laboratory collaborates closely with our illicit drugs laboratory to obtain seized drug information and conducts retrospective data analysis of potential emerging NPS and their metabolites. Once a new NPS is detected based on accurate mass and MS/MS spectrum, reference material is purchased to confirm, validate and include into routine analysis.

Results
In 2017 and 2018, the three most common NPS, in increasing order of prevalence of detection in abusers' urine specimens, were ester hydrolysis metabolites of 5-fluoro-MDMB-PICA, MMB-FUBINACA and 5-fluoro-MDMB-PINACA (also known as 5-fluoro-ADB). Many of the urine specimens were found to contain more than one synthetic cannabinoid/metabolites. When the metabolites of 5-fluoro-MDMB-PINACA were controlled in May 2018, we saw a decreasing abuse of this synthetic cannabinoid, and in Nov 2018, an analogue 4-fluoro-MDMB-BINACA appeared in the local illicit market, and the ester hydrolysis metabolite of 4-fluoro-MDMB-BINACA was detected in abusers' urine specimens. Since then, 4-fluoro-MDMB-BINACA has gradually taken over to become the most commonly abused synthetic cannabinoid in 2019. This NPS and its metabolites were controlled in Mar 2019.

Discussion

Conclusions
The fast-evolution of the NPS has posed a significant challenge in their detection, particularly in biological specimens. To deal with the challenge of identifying NPS abuse, the laboratory has developed a comprehensive drug analysis approach using High Resolution Mass Spectrometry (HRMS) with frequent updates of prevalent NPS and metabolites in the detection panels through seized drug information.
Abstract ID 491
Systematic Investigation on Matrix Effects of Neuroleptics in Plasma and Whole Blood Samples Analyzed on Three Different Mass Spectrometers.
**Julia Dinger**
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**Aim**
Analyte ionization in LC-MS/MS may be enhanced or suppressed by co-eluting analytes or matrix compounds. Such matrix effects (ME) may compromise quantitative assay performance as well as its sensitivity in case of ion suppression. Systematic ME experiments are therefore an essential part in the validation of any LC-MS/MS method. However, systematic comparisons of ME on different MS instrument types are scarce, although they may well be expected to be influenced by ion source/path design.

**Objectives:**
The aim of the present study was therefore to systematically compare ME of neuroleptic drugs in plasma and whole blood using three different MS instruments (tandem-in-time and tandem-in-space).

**Methods**
Sample analysis was performed using a commercially available LC-MS/MS kit for neuroleptic drugs in serum (kindly provided by Recipe, Munich, Germany). The precipitation reagent of the kit already containing deuterated internal standards (IS) of all 28 analytes was first spiked with different volumes of the kit’s optimization mixes (all analytes at 3 µg/mL) to obtain four different spiking reagents. Of these reagents, 100 µL were added to 50 µL of matrix samples (plasma, whole blood; n=6 each) or water to yield samples corresponding to 1.5; 15; 150; or 750 ng/mL of all analytes in matrix or water and thus covering the calibration ranges, the observed ME were completely compensated by the corresponding deuterated IS for all of the investigated analytes. For some investigated analytes, moderate differences were observed between the extent and variability of ME. However, within the respective three different LC-MS/MS instruments with respect to the analytes, moderate differences were observed between the extent and variability of ME. However, within the respective calibration ranges of the investigated matrices and LC-MS/MS instruments.

**Results**
Amisulpride, fluphenazine, melperone, norquetiapin, paliperidone, pipamperone, quetiapine, risperidone, sertindol, ziprasidone, zotepine and zuclopenthixol showed no ME regardless of matrix or instrument types and their respective optimized temperature and gas settings. Aripiprazol (QSight/QExactive) and sulpiride (QExactive/QTrap) showed ion suppression between -27% and -65% but with low variability (2-16% CV). For flupenthixol (QTrap) ion suppression (-25% to -41%) could also be observed, but with variability between 23 and 24%. Clozapin (QExactive), norclozapine (QSight), levomepromazine, perazine, promethazine (QExactive/QTrap), thioridazine and haloperidol (QTrap) showed a reproducible (≤16% CV) enhancement between +29% and +80%. In contrast, clozapine (QSight), dehydroaripiprazole (QTrap), chlorprothixene, chlorpromazine, promethazine (QSight) and prothipendyl (QTrap; 15 ng/mL) showed enhancement between +28 and +72% but with large variability between 17% and 61% CV. For these analytes, the more or less extensive ME could be compensated by the IS with the exception of dehydroaripiprazole.

**Conclusion and Discussion:**
Overall it was shown, that the extent (suppression or enhancement) and the variability of ME between samples was not different for plasma or whole blood samples for most of the investigated analytes. For some investigated analytes, moderate differences were observed between the three different LC-MS/MS instruments with respect to the extent and variability of ME. However, within the respective calibration ranges, the observed ME were completely compensated by the corresponding deuterated IS for all of the investigated matrices and LC-MS/MS instruments.

**Conclusions**

Variability in content and dissolution profiles of MDMA tablets collected in the UK between 2001 and 2018 – potential risk to users?

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Aim

MDMA (‘ecstasy’) tablets are widely used recreationally, and not only vary in their appearance, but also in MDMA content. Recently, the prevalence of high-content tablets is of concern to public health authorities.

To compare UK data with other countries, we have evaluated MDMA content of 412 tablets collected from the UK, 2001-2018, and have investigated within-batch content variability for a sub-set of these samples. In addition, we have investigated dissolution profiles of tablets using pharmaceutical industry-standard dissolution experiments on 247 tablets.

Methods

Tablets analysed were those collected from amnesty bins, or seized during entry searches, from a number of large music festivals and nightclubs in the UK. All were initially identified as potentially containing MDMA by visual identification, based on size, shape, colour and markings/logos, to tablets found to have contained MDMA previously. For MDMA content measurement, a total of 412 tablets were analysed. Included were 249 single tablets from different sources, and 45 ‘batches’ of tablets (i.e. multiple tablets from the same source, between 2 and 8 tablets per batch, total 163 tablets). The latter were used to assess within-batch variability in MDMA content. Tablets were crushed and homogenised using a clean agate pestle and mortar, and the crushed tablets dissolved in 50 % (v/v) methanol in deionised water. Solutions were analysed against MDMA calibrators (N = 6, 2 mL each calibration level), prepared by dilution of MDMA stock solution (1 g/L) over the range 0.50 – 100 mg/L in 50 % (v/v) methanol in deionised water.

For dissolution, a 7-vessel dissolution bath was used. Dissolution solvent (900 mL per vessel) was 0.05 mol/L aqueous hydrochloric acid. Bath temperature was maintained at 37 ± 0.5 °C, and the stirrer rate was 50 rpm. Samples (5 mL) were taken using a volumetric syringe via the sampling line and filter before addition of the tablet (t0), and subsequently after 5, 10, 15, 20, 30, 45, 60, 90, and 120 minutes. A final sample was drawn at 180 minutes (stirrer rate increased to 150 rpm for the final 60 minutes). After each sample was drawn, the solution was replaced with 5 mL blank dissolution solution to maintain the vessel volume. Samples drawn at each time point were collected into 15 mL screw-cap tubes and stored at 2–8 °C prior to LC-MS/MS analysis.

Results

Our data supported other studies, in that recent samples (2016 – 2018) tend to have higher MDMA content compared to earlier years. There was a statistically significant difference (p < 0.001) in MDMA content for those tablets collected pre-2010, and those collected post-2010. In 2018, the median MDMA content exceeded 100 mg free-base for the first time. Dramatic within-batch content variability (up to 136 mg difference) was also demonstrated. Statistical evaluation of dissolution profiles at 15-minutes allowed tablets to be categorised as fast-, intermediate-, or slow-releasing. These were confirmed by analysis of variance (p < 0.001) which showed three distinct groups based on the percent dissolved at 15 minutes; mean (95% CI) percent dissolved: fast-releasing 95.0 % (93.4 – 96.6 %), intermediate-releasing 68.4 % (66.5 – 70.3 %), and slow-releasing 39.4 % (37.7 – 41.1 %); and two distinct groups at 30 minutes: fast-releasing 95.7 % (94.4 – 97.1 %) and slow-releasing 57.3 % (55.2 – 59.3 %). No tablet characteristics correlated with dissolution classification. Further, within-batch variation in dissolution rate was observed.

Discussion

A number of studies have suggested that in recent years, the amount of MDMA in tablets is increasing. Data from the UK presented from the MDMA content measurement portion of this study support these observations. Furthermore, a number of batches showed marked differences in MDMA content despite similar tablet mass. For MDMA users, our data suggest that analysis of a single tablet from a batch cannot guarantee that all others in the same batch are of the same content.

The data in the second part of this study showed significant differences in dissolution profiles of MDMA tablets. These data potentially highlight an important additional risk with regards variability in MDMA tablets. There is little doubt that higher-content MDMA tablets pose a greater risk of toxicity to users than low-content tablets. Content variability aside, risks for fast-releasing and slow-releasing tablets should also be considered, especially since our data suggest no definite way to know a priori which class a tablet is likely to be in, and there were no statistically significant differences in MDMA content for fast-releasing, intermediate-releasing, or slow-releasing tablets.

Conclusions

Rapid assessment of MDMA content alone provides important data for harm reduction, but does not account for variability in (i) the remainder of tablets in a batch, nor (ii) MDMA dissolution profiles. Clinical manifestations of MDMA toxicity, especially for high-content, slow-releasing tablets, may be delayed or prolonged, and there is a significant risk of users re-dosing if absorption is delayed.
**Abstract ID 555**  
**Responding to patients and drug trends: advancement and development of clinical toxicology services at the National Rehabilitation Centre, Abu Dhabi.**  
H. E Dr. Hamad Al Ghaferi  
National Rehabilitation Center

**Aim**  
To share this unique information with the local and international addiction medicine and toxicology community, with emphasis on types of substances misused by this cohort as well as information on prevalence, patterns of substance related disorders and other insights gained from clinical experience.

**Methods**  
The NRC toxicology laboratory uses immunoassay, Gas Chromatography–Mass spectrometry (GC/MS), liquid chromatography–tandem mass spectrometry (LC-MS/MS) for the qualitative analysis of drugs with quantitative determination of some drugs as required, including buprenorphine and norbuprenorphine in serum and urine. Procedures incorporate general drug screening as well as the analysis of opiates, new (and classical) psychoactive substances, benzodiazepines, barbiturates and GHB. Methods used were chosen on the basis of proven sensitivity, selectivity, specificity and reliability. The results and findings are based on expert clinical observations at the NRC focusing on 6 years (2013 - 2018).

**Results**  
Between 2013 and 2018;  
- 56550 patient’s urine toxicology specimens were analyzed  
- 40931 samples were available for analysis by GC/MS and LC-MS/MS  
- 17148 specimens were confirmed positive for different types of drugs

The 3 most commonly encountered substances in 2013 were: Tramadol (702), Opiates (446) and Benzodiazepines (293). In 2014 the top 3 were Opiates (485), Tramadol (354) and Antihistamines & Methorphans (255). In 2015, Opiates were most prevalent (818), second was Pregabalin (Lyrica) (589) and third, Antihistamines & Methorphans (459). In 2016, Opiates were again most prevalent (1169), second was Antihistamines & Methorphans (830) and third, Pregabalin (644). In 2017 Amphetamines were most prevalent (1330), second was Opiates (943) and third, Benzodiazepines (675). In 2018, Amphetamines were again most prevalent (2379), second was Benzodiazepines (977) and third, Opiates (968). A complete list of all the substances found in the samples, types of tests and significance of the findings will be presented. Furthermore, in the past few years there has been an increasing number of samples where GHB/GBL has been detected (1 in 2017, 12 in 2018 and 42 in 2019 so far).

**Discussion**  
Over the last 6 years there has been a constant presence of amphetamines, opiates, tramadol, cannabis, benzodiazepines, pregabalin & gabapentin, antihistamines & methorphans, carisoprodol and ketamine found in patient samples. However, there have been changes in the relative frequency during this time. Trends of note though are an increase in amphetamines, a recent decrease in tramadol and frequent detection of cannabis, pregabalin, benzodiazepines and antihistamines & methorphans. The recent increased detections of GHB also require on-going monitoring.

**Conclusions**  
Drug abuse is a constantly changing scene and services need to keep abreast and modify their strategies and methodologies directed by the available information from good oversight and stewardship, clinical assessments and numerous other diversified factors of relevance supported by appropriate resources both clinically and analytically.
Abstract ID 22
The difficult interpretation of toxicological analyses in formalin-fixed tissues: a case report involving oxycodone and midazolam.

Alice Ameline
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Aim
Objective Establish the analytical and interpretation criteria when the body has been subjected to formalin preservation techniques.

Introduction
Death is accompanied by various body modifications: lividities, rigidity, cyanosis, swelling, etc. Thanatopraxy refers to all the techniques that can delay the degradation of a body and keep it in a state close to living. After having sanitized and cleaned the body, the thanatopractor incises the carotid artery and injects between six and ten liters of a biocidal product, usually composed of formalin. This operation aims to stop the invasive bacterial evolution and to slow down the decomposition. Formalin and methanol, used to stabilize the former, may change the characteristics of xenobiotics present in the body, modify the pH and dilute the compounds. The toxicological results are therefore not characteristic of the moment of death. The difficulty lies in the interpretation of the results since neither the exact volume of conservation liquid injected nor the volume of blood removed are known.

Methods
Case History The authors report the case of a 35-year-old man who died as a result of cancer. The body was embalmed the day after his death, at the request of his family, and buried. After a deeper investigation of the facts, it was envisaged that Mr. X died as a result of an overdose of oxycodone, due to a dilution error of the pump, not correctly fixed by his nurse. With this information, the body was exhumed and autopsied, 1 week after embalmment. Biological specimens were collected during the autopsy, they looked slightly watery in nature. A complete toxicological analysis was achieved within 2 weeks after embalmment.

Methods A standard analysis of blood was performed, using validated laboratory procedures. In addition, stability studies of oxycodone and midazolam were conducted in the presence of formalin to determine possible influence. A 30 mL pool of blank blood containing 1 mg/L of oxycodone and midazolam was prepared and then aliquoted into 30 tubes of 1 mL. Except for the first tube, 20 µL of 20% formalin was added to each tube. The tubes were stored at +4 °C until analysis. On day 1, the first set of tubes (without formalin) was extracted and analyzed on ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS, Waters). The stability studies were spread over 21 days, and each analysis was done three times (n = 3).

Results
The femoral blood analyzes showed the presence of methanol (1.31 g/L) but no ethanol, midazolam at therapeutic concentration (74 ng/mL) and oxycodone at a toxic concentration (152 ng/mL). No other compound was detected. It can be concluded that the concentrations measured in blood were certainly lower than those at the time of death, without being able to evaluate the percentage of degradation and/or dilution. The stability study of oxycodone showed a rapid and complete degradation of the molecule, in contact with formalin (-99% in 9 days). Midazolam was less affected, although instability was evident (-48% and -73% in 9 and 21 days, respectively). These results should be taken into account when interpreting the concentrations.

Discussion
The results of the stability studies prove the degradation of both midazolam and oxycodone in the presence of formalin. It is therefore assumed that the victim was exposed to higher concentration of oxycodone, confirming the mistake of the nurse. This was also documented when evaluating the oxycodone pump records. The concentrations obtained during the toxicological analyzes are therefore underestimated in relation to the concentrations at the time of death, but the level of variation cannot be quantified.

Conclusions
Formalin preservation techniques have negative consequences for toxicological analyses and their interpretations. Stability studies of oxycodone and midazolam were instrumental in understanding the probable causes of death of Mr. X. These observations constitute the first stability studies of these two compounds in the presence of formalin. Declaration of conflict of interest: The authors declare no conflict of interest.
Abstract ID 57
Statistical validation of changes in the rate of acute fatal alcohol poisonings.

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Aim
Alcohol (ethanol) is a major cause of mortality and morbidity in Finland and worldwide. Determining the cause of death in cases with a history of excess drinking is not always a simple task. In addition to causing death directly by acute fatal alcohol poisoning, alcohol may induce illnesses or accidents that lead to death. Signs of alcohol induced illnesses, such as liver cirrhosis, may well be present even in acute alcohol poisoning deaths. Thus, toxicology – and in particular determining the alcohol concentration in different post-mortem matrices – has an important role in determining the underlying cause of death in such cases.

The number of fatal alcohol poisonings in Finland has decreased greatly in the last 10 or so years. The aim of this study was to investigate whether this trend is real or an artefact caused by possible changes in the process of determining the cause of death in alcohol-related fatalities. In an effort to assess this, changes in the median blood alcohol concentration in fatal alcohol poisonings over this time period were examined. In addition, changes over time in the age and gender ratio of the deceased were studied.

Methods
The national post-mortem toxicology registry was utilised for the assessment of all post-mortem cases in which the underlying cause of death was fatal alcohol poisoning in a period of 32 years (1987–2018) in Finland. Number of cases per year, blood alcohol concentration (BAC), and age and gender of the deceased were assessed. In addition, we compared the number of fatal acute alcohol poisonings to the number of deaths from alcohol induced illness in a period of 10 years (2008–2017). The ICD-10 codes that were used to extract the alcohol induced illness cases from the toxicology database included F10.1–F10.9, G31.2, G40.51, I42.6, K29.2, K70, and K86.0.

Results
In the 1987–2018 study period, there were a total of 12,328 fatal acute alcohol poisonings. For the first 15 years, the number of fatal acute alcohol poisonings remained relatively stable, around 400 cases per year. After that, the yearly number rose to over 500 cases until a long and steep period of decreasing trend started at 2004. Between 2004 and 2017 the number of fatal acute alcohol poisonings decreased 59%, from 545 to 223 cases per year. At the same time the number of alcohol induced illnesses remained stable or decreased only slightly.

The median BAC in all acute fatal alcohol poisonings was 3.2 g/kg. There was a slight overall decrease in the median BAC over the study period from around 3.4 to 3.1 g/kg (p<0.01). The median BAC in alcohol induced illness deaths was 1.0 g/kg. In 5% of the illness deaths BAC was above 2.5 g/kg.

The proportion of women in fatal alcohol poisonings increased over the study period from around 16% to nearly 25%. The mean age of the deceased increased significantly throughout the study period from 47.7 years in 1987 to 56.6 years in 2018 (p<0.001). The mean age of females was higher than that of males in all of the studied years.

Discussion
Finland has long been known for its heavy drinking culture. However, in recent years the drinking habits have undergone major changes and as a result the number of fatal acute alcohol poisonings has decreased markedly.

The decrease in the number of acute fatal poisonings was not accompanied by an increase in deaths due to alcohol related illness. Therefore, the decrease cannot be due to changes in the categorization of specific cases as one cause of death versus the other. It could be speculated that the decrease in the median BAC might be related to the increasing age of the victims of fatal alcohol poisoning since older age is associated with stronger effects of alcohol on the human body as well as with extensive use of drugs that may further exacerbate the effects of alcohol.

The increasing mean age of the victims of fatal acute alcohol poisoning reflects the fact that the decrease in the number of such cases is concentrated in younger age groups. Whereas the number of fatal acute alcohol poisonings has decreased steeply among males of all ages, the number for females has been stable resulting in an increase in the proportion of women among the victims of fatal acute alcohol poisoning. This is well in line with previous research that has shown that gender differences in the alcohol use patterns and the related outcomes have become narrower in recent years [1].

Conclusions
In this study we showed that the decrease in the number of fatal poisonings in Finland in recent years is not a result of changes in the practice of determining fatal poisoning but rather a real phenomenon that likely reflects changes in the overall consumption of alcohol.

Abstract ID  62
Katherine Wong

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Aim
Tramadol is a synthetic opioid prescribed for moderate to severe pain relief in adults and in children >12 years old. As well as producing an analgesic effect, tramadol enhances serotonin release and inhibits neuronal uptake of noradrenaline. Despite limited research on the overall efficacy of tramadol for paediatric patients, it is being increasingly used in Australia for children of all ages; this is contrary to other health agencies, such as the US Food & Drug Administration (FDA)[1], stating that tramadol should not be used to treat pain in children.

This is a case report involving the death of a young child after an adenotonsillectomy in hospital and a subsequent accidental overdose of tramadol. The discussion of the case aims to highlight the significance of toxicology findings and the importance of the Coroner’s role in death investigation.


Methods
In Victoria, Australia the role of the Coroner is to investigate unexpected deaths to determine the identity of the deceased, the cause of death and, in some situations, the circumstances surrounding the death. The Coroner will also seek to identify and make recommendations on how similar deaths may be prevented in the future. The population of Victoria is ~6m with ~39000 deaths reported each year; of these ~6500 (17%) are referred to the coroner, of which ~5800 cases receive toxicology analysis. Toxicological analysis consists of screening and quantification for alcohol (GC-FID) and a comprehensive range of prescription and illicit drugs (tandem LC/MS/MS) in preferably femoral post-mortem or ante-mortem blood and other associated post-mortem specimens and/or exhibits.

Tramadol was extracted by a basic liquid-liquid extraction, followed by confirmation and semi-quantitation by LC/MS/MS (parent mass of 264.2; two product ions m/z 57.1 and 58.1) using a fully validated method according to international guidelines.

Results
Tramadol had been prescribed to the child as an analgesic upon discharge from hospital post-adenotonsillectomy. The dose of tramadol was 25 mg to be given 4 times per day, as per manufacturer’s recommended dosage guidelines. Following the last dose of tramadol the child was found unresponsive, re-admitted to hospital and later died despite intensive care. An ante-mortem blood sample belonging to the child that was acquired by the hospital 20 hours after last administration revealed a tramadol concentration of 1.4 mg/L.

Discussion
The concentration of tramadol detected in the ante-mortem blood sample was determined to be elevated compared with the stated dose and time of administration. In the absence of any other autopsy findings, the cause of death was given as tramadol toxicity consistent with excessive administration.

The formulation type, method of administration, and information provided to the parents regarding side effects and risks of Tramadol for children were assessed by the Coroner. The tramadol in this case had been supplied as Tramal® Oral Drops - a formulation which was not approved for use in Australia for children less than 12 years of age. As a result of the Coroner’s investigation recommendations were made for both the Therapeutic Goods Administration and the prescribing hospital. These recommendations have led to improved procedures and systems at the hospital, including a patient information sheet, and revision of the internal protocol for post-operative management and the standardized analgesic ladder.

Conclusions
This case highlights the significance of toxicology findings and the importance of the Coroner’s role in death investigation. It provides an example of how coronial toxicological analyses can provide answers for families and inform strategies for future death prevention.
Abstract ID 87
Can cannabis really kill you?
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Aim
Synthetic cannabinoids have caused a large number of emergency presentations to hospitals for adverse cardiovascular events including numerous deaths, particularly for the more potent analogs acting on the CB1 receptor. While smoked cannabis use is often associated with significant changes in heart rate and cardiac output, amongst other physiological changes, it has been rarely considered in the forensic literature as a significant contributory or causal factor in sudden unexpected death. A review of case reports of admissions to hospitals for cardiovascular events was undertaken together with a review of epidemiological studies, and case reports of sudden death attributed, at least in part, to use of this drug.

Methods
All publications in the English language where cannabis was believed to have contributed to an admission to an emergency department at a hospital for a cardiovascular or related medical event, or was believed to have contributed to, or caused, the death were reviewed.

Results
Thirty seven publications were reviewed and showed that use of cannabis is not without its risks of occasional serious medical emergencies and sudden death, with reports of at least 35 persons presenting with significant cardiovascular emergencies who had recently smoked a cannabis preparation.

Discussion
At least 13 deaths from a cardiovascular mechanism have been reported from use of this drug which is very likely to be an under-estimate of the true incidence of its contribution to sudden death. In addition, many cases of stroke and vascular arteritis have also been reported with the latter often involving a limb amputation. There was no obvious relationship between blood concentration of THC (or metabolite) and outcome. The age ranges varied from 17-52 years old and there were no other drugs detected in all of these selected cases.

Conclusions
While it is a drug with widespread usage among the community with relatively few deaths when faced with a circumstance of very recent use (within a few hours), a positive blood concentration of THC and a possible cardiac-related or cerebrovascular cause of death this drug should be considered, at least, as a contributory cause of death in cases of sudden or unexpected death. This presentation will include some representative case reports to illustrate the circumstances, pathology and toxicology.

Keywords: cardiovascular disease, myocardial infarction, terpenoids, THC.

Abstract ID 163
A French case report of carfentanil fatality including blood, urine and hair investigations.
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Aim
Carfentanil (CaF) is a new synthetic opioid (NSO) solely approved for veterinary use (to incapacitate various species of large exotic wildlife) as it is 10,000 times more powerful than morphine. CaF has wildly recently appeared emerged in the NPS market and many overdose deaths have been reported in the USA since 2016. In December 2017, a 41-years-old polydrugs abuser for several years, especially of NPS purchased on the darkweb, is found dead in his room, a syringe planted under the tongue. His history includes recent CaF intoxication treated with Nalscue®. The Prosecutor requires an autopsy and toxicological analyzes

Methods
Autopsy specimens (blood and urine) were analysed using standard laboratory techniques (GC-FID, GC-MS and LC-MS/MS). A LC-HRMS method was used to perform a broad and specific search for NSOs including CaF and metabolites in blood, urine and hair samples

Results
CaF was identified in blood and urine (respectively 4.2 and 0.4 µg/L). Other illicits drugs were identified in blood and urine including morphine (respectively 4.5 and 10.2 µg/L), ketamine (respectively 2.2 and 2.2 µg/L), metamphetamine (respectively 7.2 and 50 µg/L), amphetamine (12 µg/L in urine) and THC (1.2 µg/L in blood). Various pharmaceuticals were also identified including methadone and EDDP (111 and 23 µg/L in blood; 521 and 503 µg/L in urine), alprazolam (22 µg/L in blood; 175 µg/L in urine), zolpidem (19 µg/L in blood; 9 µg/L in urine) and dextromethorphan (0.8 µg/L in blood; 2 µg/L in urine). In addition, these drugs were also identified in hair sample (brown, 9 cm length) together with additional drugs: other NSOs including benzoylfentanyl (concentrations ranged from 621 to 5870 pg/mg) and 4-fluorobutyrylfentanyl (concentrations ranged from 4 to 719 pg/mg), other NPS (ethylhexedrone, dicyazepam, methoxetamine and synthetic cannabinoids: AB-Fubinaca, MAM 2201), illicit drugs (heroin, cocaine, MDMA) and pharmaceuticals (cyamemazine, codeine, diazepam, quetiapine, sildenafil). Observed CaF hair concentrations ranged from 54 to 166 pg/mg in the three 3-cm length hair segments.

Discussion
CaF use is here associated with other opioids, illicit drugs and psychotropic depressants of the respiratory system, like the other cases described in the literature. Blood concentration of CaF is of the same order or higher than those reported in cases of lethal poisoning described in the USA and recently in UK. To our knowledge, this is the first identification of this fentanyl derivative in a fatality in France

Conclusions
Although hair results strongly suggest a significant tolerance of the victim to NSOs, the relatively high post-mortem CaF concentration observed in the victim’s blood (4.2 µg/L) together with the absence of metabolite (especially NorCaF) in blood or urine, suggest a sudden death immediately following a significant CaF dose intake (presumably injected).
Abstract ID 172
Qualitative and Quantitative Analysis of Cyanide in Blood by Headspace Gas Chromatography- Mass Spectrometry.
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Aim
Cyanide salts, mainly Sodium Cyanide (NaCN), and Potassium Cyanide (KCN) are used in homicidal and suicidal poisoning in Sri Lanka due to their ready availability & easy accessibility. As a result, Government Analyst’s Department receives numerous such cases for forensic analysis which require a highly reliable method. The aim of this study was to develop a simple, rapid and sensitive method with high accuracy for the quantitation of cyanide in blood using Headspace Gas Chromatography- Mass Spectrometry (HS-GC/MS).

Methods
Cyanide in blood was liberated as the volatile hydrogen cyanide by acidification in a headspace vial using Agilent 7697A Headspace auto-sampler. The liberated hydrogen cyanide was analysed by Agilent 6890 Series gas chromatograph coupled with Agilent 5973N mass selective detector using a GS GASPRO column (60 m length × 0.32 mm ID). Sample: Acid ratio to be used in sample preparation, Headspace - oven, loop & transfer line temperatures, equilibration time and the GC temperature program were optimized and developed a sensitive and reliable method for the analysis of cyanide in blood in forensic cases. The developed method was validated and suitability was statistically compared with the results obtained from the spectroscopic method using 1, 3-dimethylbarbituric acid by applying t-Test. The developed method was successfully applied in the analysis of samples received by the Forensic Toxicology Laboratory.

Results
The optimization results revealed that the best suitable Sample : 5N Sulphuric acid ratio is 1:1. While the best suitable headspace condition to be applied in the analysis of cyanide in blood was found to be oven, loop and transfer line temperatures of 90 OC, 95 OC & 100 OC respectively. Under the above conditions, the optimum thermostat time was found to be 10 minutes. The optimized oven temperature program in GC was initially 50°C and increased by 40 OC/min up to 150°C. The developed method showed a simple chromatogram with a peak of HCN at the retention time of 8.346 min. This method showed good linearity (r2) where, Correlation coefficient value for 2.0 – 100.0 µg/ml of HCN in blood fortified with cyanide and aqueous cyanide solution were 0.997 and 0.999 respectively. Therefore, the developed method has a linear dynamic range from 2.0 - 100.0 µg/ml for postmortem specimens. The limit of detection and the limit of quantification of cyanide were 2.25 µg/ml and 2.77 µg/ml respectively. The percentage recovery and the precision (%RSD) of the method at six different concentrations of 4.0, 6.0, 10.0, 20.0, 60.0, 100.0 µg/ml of HCN were in the range of 83.1% - 96.0% and 1.70% – 3.05% respectively.

Suitability of the spectroscopic and HS-GC/MS method was evaluated with the recoveries where the recoveries of the two methods at Cyanide concentration in blood 10 µg/ml were 53.6% and 90.7% respectively. Further, the two mean concentrations obtained in the analysis of ten replicates at the concentration of Cyanide in blood at 10 µg/ml using the two analytical methods were significantly different at the chosen 95% significance level by means of the t-test indicating that the values for concentration of HCN from blood spiked samples reported by the two analytical methods are significantly different.

Twenty blood samples sent by the Judicial Medical Officers in suspected cyanide cases either suicide or homicide were analysed. Out of these twenty blood samples eleven were positive and the HCN concentrations were in the range of 2.8 – 45.4 µg/ml.

Discussion
In the spectroscopic method the low recovery may be attributed to loss of HCN during distillation. Further, spectroscopic method has been reported to have interference from thiocyanate, nitrate, thiosulfate, etc. that can result in both positive and negative bias. However, in the HS-GC/MS method the above problems are totally eliminated and % recovery increased to 90.7%. This technique is rapid, require low analytical sample volume and minimizes the formation of artifacts and can accurately quantify volatile substances. In the developed method peak area of HCN decreased in proportion to volume of 5 N sulphuric acid, that may be due to rapid loss of HCN when more sulphuric acid is added to the vial. The equilibration time used in the literature were between 15 – 60 minutes, in the developed method it was reduced to 10 minutes.

Conclusions
Present study describes a reliable, specific and sensitive method that requires no analyte derivatization or special sample pre-treatment. Suitability of the spectroscopic and HS-GC/MS method was evaluated by means of the t-test and found that the developed HS-GC/MS method is highly suitable for the forensic toxicological determination of lethal levels of cyanide in blood. The simplicity and short analytical time make this method more favorable than previous analytical techniques available for the analysis of cyanide and avoid interference arise in the spectroscopic method.
Abstract ID 183
Recognition of drugs showing exceptionally high or low median concentration levels in post-mortem femoral blood.
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Aim
Reference concentrations of drugs play a major part in the interpretation of analytical toxicology results. However, post-mortem (PM) drug concentrations are not necessarily the same as those at the time of death. Published case notes or small case series, as well as compilations of PM concentrations collected from literature sources are available for many drugs, but these data are often heterogeneous in terms of the sampling site, the analytical methods used, and the number of cases involved. As the medico-legal autopsy rate in Finland is high and the manner of death is natural in approximately 55% of all PM toxicology cases, toxicologically relevant estimates can be calculated for normal and toxic concentrations. In this study, our objective was to recognize such drugs that show an exceptionally high or low median (‘normal’) concentration level in PM femoral blood, as compared to clinical therapeutic range, in cases involving all causes of death.

Methods
During an 18-year period, the results from quantitative analyses of drugs in PM femoral venous blood from 122 234 autopsy cases in Finland were entered into the toxicology database. From this material, those 183 drugs and metabolites that had been quantified at least 18 times in PM blood were selected, and the median, mean, and upper percentile (90th, 95th, 97.5th) concentrations (mg/L) were calculated from the findings. For each drug, the ratio of the median drug concentration in PM blood to a literature value in living persons’ plasma (Cplasma) was calculated to get a sense of the magnitude of PM change. In most cases, Cplasma values represented the upper limit of plasma/serum concentrations observed following therapeutically effective doses. The drugs showing an exceptionally high or low PM median/Cplasma ratio were selected for further investigation.

Results
The proportion of fatal drug poisonings in the present material was on average 8%. For 76 drugs (42%), PM median/Cplasma was between 0.5 and 1.5, suggesting that for those drugs the PM median concentration was comparable to the clinical plasma levels. For 41 drugs (22%), the ratio was higher than 1.5, and for 62 drugs (33%) the ratio was below 0.5. The fifteen drugs showing the highest PM median/Cplasma ratios (≥ 2.5) included hydroxychloroquine, promazine, levomepromazine (methotrimeprazine), dextromethorphan, desloratadine, metformin, paroxetine, amlodipine, memantine, chlorpromazine, doxepin, fluvoxamine, bupropion, donepezil and olanzapine. The fifteen drugs showing the lowest PM median/Cplasma ratios (≤ 0.1) included pioglitazone, salicylate, oxazepam, diazepam, valsartan, quinine, naloxone, losartan, lorazepam, lidocaine, ketamine, hydrochlorothiazide, glimepiride, clonazepam and carvedilol.

Discussion
Several explanations can be found for these exceptionally high or low PM median/Cplasma ratios. The most obvious pharmacological reason for a high or low ratio is a high or low volume of distribution (Vd), respectively. In the high PM median/Cplasma group, the Vd was ≥ 20 L/kg and/or the elimination half-life was long. In the low PM median/Cplasma group, the Vd was always ≤ 4 L/kg. The concentration statistics of promazine, levomepromazine and doxepin can be skewed due to the fact that more than 20% of the cases were fatal poisonings, raising the PM median. An artificially high PM median may also be due to a limit of quantification level inappropriately high for the drug in question. Metformin is a special case as this drug exhibits complex pharmacokinetics with occasional largely elevated ante-mortem concentrations and a high blood-to-plasma partition ratio. Finally, the representativeness of the Cplasma literature values plays a key role concerning the relevance of the PM median/Cplasma ratio.

Conclusions
Evidence based information about drug reference concentrations is essential for the interpretation of PM toxicology cases. Knowing the ‘normal’ PM concentration level and the reasons for PM change helps the toxicologist and pathologist to avoid pitfalls associated with drugs showing exceptionally high or low concentrations. However, the concentration information should always be interrelated with pharmacological and toxicological considerations, the case history, and autopsy findings.
Abstract ID 187

Postmortem redistribution finally made predictable by a metabolomics approach? A proof-of-concept study involving morphine and methadone cases.

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Aim
Postmortem redistribution (PMR) is a well-recognized phenomenon leading to difficulties in interpretation of postmortem cases in a forensic toxicological context. Systematic studies so far focused on direct measurement of time-dependent postmortem concentration changes of centrally acting substances to assess the potential of a substance or substance class to undergo PMR. The novel approach of the current study, however, utilizes metabolomics techniques (the qualitative and quantitative characterization of small endogenous molecules such as amino acids, fatty acids and sugars) to find endogenous surrogate analytes as potential indicators of PMR for specific substances or substance classes. The aim of this proof-of-concept study was to assess whether or not these endogenous surrogates allow prediction of PMR of morphine and methadone; model compounds of high interest in forensic toxicology.

Methods
For postmortem cases involving morphine (n=20) and methadone (n=12), femoral blood samples were collected at two time points; at admission of the deceased to the institute (t1) utilizing a computed tomography guided biopsy tool connected to a robotic arm and manually approximately 24 h later (t2) during the medico-legal autopsy. Samples were analyzed with GC-MS (Q Exactive GC Orbitrap) after protein precipitation (methanol), manual methoxyimation (methoxyamine HCl in pyridine) and on-line silylation (N-Methyl-N-trimethylsilyl trifluoro acetamide (MSTFA)). Data acquisition was untargeted, but for quantification purposes, targeted data processing was performed for 59 endogenous metabolites (including amino acids, fatty acids, sugars and organic acids). Additionally, all femoral blood samples have previously been analyzed for their morphine or methadone concentration by LC-MS/MS (5500 QTrap) using a validated method. Statistical analysis was carried out to find correlations between the time-dependent concentration changes of endogenous compounds and morphine or methadone, respectively.

Results
Morphine and methadone showed significant concentration increases in femoral blood over time within the studied time-frame. Spearman’s rank-order correlation analysis found statistically significant (p < 0.05) negative correlations between time-dependent concentrations changes of morphine with creatinine, glutaric acid, hypoxanthine, fructose, pentadecanoic acid, palmitoleic acid and alanine (with correlation coefficients of -0.6346, -0.5970, -0.5955, -0.5444, -0.5338, -0.5203 and -0.4782, respectively). Concentration changes of methadone over time were found to be statistically significantly correlated with time-dependent concentration increases of its metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP; spearman’s rank correlation coefficient: 0.7902) and with one of the targeted endogenous compounds (hexadecanoic acid; spearman’s rank correlation coefficient: 0.5545).

Discussion
Statistically significant correlations, positive and/or negative, between time-dependent concentration changes of a drug and endogenous metabolites might indicate a parallel respectively an opposite postmortem behavior in the human body. Hence, making use of these similarities/dissimilarities, quantification of selected endogenous metabolites in parallel to the quantification of a drug within a single postmortem case sample seems to be a promising tool to aid in the routine assessment of PMR, despite inter-individual variability.

Conclusions
The current study serves as a proof-of-concept that a metabolomics approach can be successfully applied to systematic PMR assessment, finally serving the aim to make forensic toxicological postmortem case interpretations more reliable.
Abstract ID 193
Detection of New Psychoactive Substances (NPSs) in Dried Blood Spots (DBSs) collected from postmortem cases.
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Aim
Blood collected and dried on a paper substrate (i.e., “dried blood spots” or DBS) is an alternative simple method of sampling and storage that allows to obtain reliable analytical results even after a long period from collection time. Nowadays, several hundred compounds are included in the group of New Psychoactive Substances (NPS); hence, it is difficult for a laboratory to purchase all the standards and to develop and optimize analytical procedures for the detection of these molecules in biological specimens. The aim of this study was to develop a target screening LC-MS/MS approach, based on parameters provided by previously published studies, for the detection of New Psychoactive Substances (NPS) in DBS collected from postmortem cases, and to compare the results obtained with and without reference standards.

Methods
85 µL of blood were deposited on a commercial card for DBS analysis. The whole blood stain was cut and put in a plastic tube, containing 1 mL phosphate buffer at pH 6 and 11 different deuterated internal standards at the concentration of 200 ng/mL. Samples were analyzed within 24 hours after blood deposition on the paper substrate. Stability experiments have not been performed. The sample was sonicated for 10 minutes and centrifuged. Supernatant solution was purified on a Bond Elut Certify solid phase extraction (SPE) cartridge, dried under nitrogen stream, and reconstituted in 200 µL mobile phase; finally, 5 µL were injected in the LC-MS/MS system. The analytes were separated through a reverse phase chromatography on a C18 column and detected on a triple quad operating in Multiple Reaction Monitoring (MRM) mode and positive polarization, monitoring two transitions for each analyte. A first LC-MS/MS screening was set up by achieving MRM parameters from 20 previously published articles, giving priority to studies that used the same triple quad that was available in our lab, together with similar chromatographic conditions. A total of 120 substances, among synthetic cannabinoids (72), substituted phenethylamines (26), synthetic opioids (22), were included in the list of monitored compounds, and the qualitative screening method was applied to 80 postmortem cases, collected by the lab within a 4-year-period. 40 reference standards (30% of the total number) have been eventually purchased and the 80 DBS cases were re-evaluated, after validation of the analytical procedure.

Results
The method is simple and relatively fast. Among 80 cases, 8 different compounds were initially identified by the literature-based screening approach: 2 different NBOMes (25H-NOBOMe and 25C-NBOMe), 3 fentanyl analogues (furanyl fentanyl, 4-ANPP and acryl fentanyl) and 3 substituted phenethylamines (α-PHP, 3,4-dimethylmethcathinone, 4-fluoromethcathinone). Eventually, the method has been validated on 40 out of 120 substances, including the molecules that provided positive results, and the 80 cases have been re-evaluated accordingly. The presence of 5 out of the 8 NPS (25H-NOBOMe and 25C-NBOMe, furanyl fentanyl, 4-ANPP and α-PHP) have been confirmed through the validated method; yet, the concentration of 25H-NBOMe was found to be lower than 1 ng/mL, and the concentration of 25C-NBOMe and furanyl fentanyl lower than 3 ng/mL, indicating an appropriate sensitivity of the screening method. The identification criteria for acryl fentanyl, 3,4-dimethylmethcathinone and 4-fluoromethcathinone were not achieved and the results have been considered as false positive. Among the 40 substances evaluated after the validation of the method, the analytical procedure did not reveal any false negative results.

Discussion
A target screening method for the detection of several dozen NPSs in DBS has been successfully developed. Preliminary results on 80 authentic positive postmortem cases suggested that DBS collection during autopsy is of great interest and could be a good, simple, reliable and complementary method of sample storage. Though the validation of a method through the use of reference standards remains mandatory in the forensic field, the results obtained from this preliminary study support the transferability of MRM parameters between different triple quads, by guaranteeing the detection of different compounds in DBS, even at low concentrations. Yet, a limited number of false positive results assesses the effectiveness of the initial analytical approach. So far, no false negative results have been detected.

Conclusions
Literature-based target LC-MS/MS screening of NPSs on DBSs provided to be a reliable and sensitive analytical approach whether reference standards are not promptly available. The absence of false negatives should be now assessed for the whole group of monitored compounds.
Abstract ID 270
The Trouble with Kratom: Analytical and Interpretative Challenges involving Mitragynine.
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Aim
Mitragynine is the primary active alkaloid in the leaves of the tropical tree Mitragyna speciosa, and goes by the popular names “Kratom”, biak-biak, and maeng da. Mitragynine is increasingly seen in forensic toxicology casework including driving under the influence of drugs (DUID), and medicolegal death investigation cases. The toxicity of mitragynine continues to be debated in the scientific community as advocates highlight its long history of use in Southeast Asia and testimonials to its benefits by present day users, while opponents point to an increasing number of adverse events tied to mitragynine use in Western societies. Quantitative reports of mitragynine in biological specimens from forensic investigations in the literature are sparse and may be influenced by poor analyte stability and inadequate resolution of mitragynine from its diastereomers, which could lead to falsely elevated concentrations and subsequently render those reported concentrations inappropriate for comparison to a reference range. We describe the application of a method capable of resolving mitragynine from its diastereomers, and its application to the analysis of postmortem and human performance casework specimens.

Methods
Over the course of 27 months, 1001 blood specimens submitted to our laboratory tested positive for mitragynine using a sensitive and specific quantitative LC-MS/MS method. Quantitative analysis for mitragynine was performed using a Waters TQD® Tandem Mass Spectrometer with a Waters Acquity® Ultra Performance LC system (Waters Corp.®, Milford, MA). The mass spectrometer was operated in ESI positive ionization mode with a capillary voltage of 0.5 kV. The source temperature was 150°C and the desolvation temperature was 450°C. The chromatographic separation was achieved with a Thermo Scientific® BETASIL Silica-100, 2.1 x 100mm column with a 5.0-micron particle and EXP filter cartridge. An isocratic method of 10% ammonium formate buffer, pH 4.0 to 90% acetonitrile was used to separate mitragynine from its diastereomers. The total method run time was 3.5 minutes.

Results
From the 1001 samples tested, concentrations ranged from 5.6-29,000 ng/mL, with mean and median concentrations of 410 ± 1124 and 130 ng/mL respectively. During validation it was documented that mitragynine stability declined markedly after 30 days, thus, any analytical result obtained more than 30 days after specimen collection may underestimate the actual mitragynine concentration. The longer the period between specimen collection and testing, the greater the decrease from its original concentration.

To obtain a better understanding of mitragynine concentrations in postmortem cases that are least likely to have suffered loss resulting from a delay between specimen collection and testing, the mitragynine cases were sorted to identify postmortem cases in which the case was reported within 35 days of specimen collection. The concentration range for the 583 postmortem cases where mitragynine was analyzed within 35 days of specimen collection was 5.9-4,400 ng/mL, with mean and median concentrations of 372±594, and 140 ng/mL respectively. Since early 2018, when quantitative testing was made available, mitragynine was detected in DUID populations in 20 cases out of approximately 17,500 submissions. Of the 20 cases, which were submitted for analysis <35 days from the time of specimen collection; the concentration range for these cases was 11-490 ng/mL, with average and median concentrations of 106±117 and 75 ng/mL.

Discussion
Mitragynine is increasingly being detected in postmortem cases but rarely seen on its own. These cases appear to be separated between recreational poly-substance cases and potentially chronic pain management cases. Mitragynine is frequently encountered with other opioids, which then makes it exceedingly difficult to determine the effect mitragynine had on a case relative to another opioid. Although opioids are typically the most frequently encountered drug class with mitragynine, the same difficulty with interpretation will occur with other CNS depressants such as benzodiazepines. When evaluating the frequency of reported concentrations, 81% of cases had a result of less than 500 ng/mL, with 92% of cases less than 1000 ng/mL.

Although each case has to be evaluated on its own merits since drug concentrations vary widely and are affected by a large number of variables such as route of administration, time of sample collection, stability of drug in a sample, accuracy of laboratory measurement and poly-pharmacy, mitragynine concentrations between 100-500 ng/mL may need to be scrutinized as contributory, while concentrations >1000 ng/mL are more frequently being associated with fatalities and may be more causative in nature.

Conclusions
Mitragynine presents an analytical challenge that requires a method which appropriately separates and identifies mitragynine itself from its isomers and other related natural products. We describe a validated analytical method and present a short series of case reports that provide examples of apparent adverse events and the associated range of mitragynine concentrations. It is only with this type of analytical specificity that guide interpretation of mitragynine concentrations in biological specimens in forensic casework, and assess its potential toxicity.
Investigation into the role of alcohol and cocaine in violent suicide.

Sue Paterson

Abstract ID 319

Aim
In the UK suicide is the leading cause of death for men under the age of 49 and women under the age of 34. Both alcohol and alcohol dependence are associated with an elevated risk of suicide and there is evidence that cocaine use is also a significant risk factor. However, data currently available in the UK on substance use in suicide is limited to patients in contact with secondary psychiatric services which accounts for only 28% of suicide deaths. Alcohol-associated suicide, apart from self-poisoning with alcohol, is not included in official statistics on alcohol-related deaths. Accurate data concerning the extent of alcohol and cocaine use in suicidal deaths would be useful for suicide prevention strategies which have previously given little attention to patterns of drug use.

The aim is to investigate the extent of alcohol and cocaine use in cases of suspected violent suicide by analysis of a large post-mortem toxicological database in order to get a more accurate estimation of the involvement of both in violent suicide.

Methods
The Toxicology Unit, Imperial College London performs toxicological analysis for coroners in London and SE of England covering approximately 15% of England’s population. The unit’s database from 01.01.2012 to 31.12.2016 was searched for cases where death was due to violent suicide. This included cases of hanging, carbon monoxide poisoning, asphyxiation, jumping in front of a train, laceration, gunshot wound and immolation when the act appeared deliberate and self-inflicted. Cases with insufficient evidence to conclude suicidal intent, for example unwitnessed train-related deaths, were excluded.

Blood alcohol concentration (BAC) was measured by dual column headspace GC-FID. A BAC greater than 50 mg/100ml was considered positive.

The presence of cocaine in the blood was determined from the general screen for drugs and the presence of cocaine and/or benzoylecgonine (BE) in the urine was determined from the screen of urine for drugs of abuse, both screens were carried out using full scan GC-MS.

Results
Of the 10,287 cases searched, 1,722 were determined to have died by violent suicide. Most decedents were male (1,398) with a median age of 43 years and 324 were female with a median age of 41 years. Hanging was the most common form of violent suicide with 1,053 male and 252 female, followed by train deaths 123 male, 25 female; asphyxiation 89 male, 35 female; laceration 67 male, 7 female; gunshot wound 20 male, 0 female; immolation 13 male, 2 female.

Of the 1,722 cases of violent suicide identified, BAC was found to be ≥50 mg/100ml in 507 (29.2%). The breakdown of alcohol concentration was: 50-99 mg/100ml, 117 male, 12 female; 100-199 mg/100ml, 192 male, 41 female; 200+ mg/100ml, 125 male, 20 female.

Cocaine and/or BE were present in 144 (8.4%) of the total cases of violent suicide and in cases of hanging, the most common form, 123 (9.5%). Cases positive for cocaine and/or BE were significantly more likely to have a BAC ≥50 mg/100ml (P≤0.0001).

Our results showed that increasing concentrations of alcohol increased the likelihood of testing positive for cocaine and/or BE in a dose dependent fashion up to concentrations consistent with severe intoxication/unconsciousness.

Discussion
In summary, we found a BAC ≥50 mg/100ml in 29.2% and cocaine and/or BE present in 8.4% of all suspected violent suicides. Cocaine use in those using alcohol rose to 16.6%. The Office for National Statistics reports that 2.3% of adults aged 16 to 65 have used cocaine in the last year and only 0.3% of are frequent users which demonstrates that cocaine use is far more prevalent in suicides than in the general population. This suggests that clinicians should ask about alcohol and cocaine use in suicidal patients.

When the stimulatory effects of cocaine have finished a period of depression occurs and suicidal ideation is associated with this depressive phase. Therefore, to get a more accurate estimation of cocaine use in violent suicide it would be useful to investigate chronic rather than acute use. This could be achieved by analysis of hair samples.

Conclusions

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Baclofen: to screen or not to screen?

Limon Nahar

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Aim

Baclofen (BLF) has been clinically prescribed in the UK since 1972 for the alleviation of spasticity due to multiple sclerosis. BLF is also currently being investigated as a treatment for alcohol dependence and the withdrawal symptoms of gamma-hydroxybutyrate (GHB) use. The General Medical Council (UK) has already approved its off-label use for treating alcoholism. However online surveys conducted in the UK and from a study from 16 emergency departments across 10 European countries suggest BLF has recreational misuse potential. Anecdotal evidence on online forums regarding the recreational use of BLF suggest that at very high doses BLF can provide similar effects to GHB. Individuals have stated that combining BLF with pregabalin (PGL) or gabapentin (GBP) provides enhanced euphoria.

With current post-mortem (PM) toxicological screening approaches, BLF is not routinely included in the general drugs screen and is only screened for if it is mentioned in the case documents. To date there has been no data reported on the prevalence of BLF in PM Coroners’ toxicology.

The aim of this study is determine the prevalence and concentrations of BLF in Coroners’ toxicology to establish whether or not there is a requirement to screen for BLF in all PM cases. To determine whether BLF misuse with ethanol (≥ 50 mg%), GHB, PGL and or GBP was causing death and the potential extent of the under-reporting of BLF-associated deaths to the Coroners due to screening approaches which do not routinely include BLF.

Methods

All PM femoral blood samples (aged 16 years and over) received at the Toxicology Unit, Imperial College London, between 01.01.2016 to 31.12.2017 from Coroners’ cases were screened for BLF in addition to a routine screen. BLF was quantified in all positive cases. The method used was protein precipitation followed by liquid chromatography-tandem mass spectrometry.

Results

3750 PM blood samples were screened and only 21 were positive for BLF (0.6%); 11 were male and 10 were female (median age 47 and 52 yrs, respectively). Of the 21 BLF positive cases, 9 did not mention BLF in the case documents. This means, if a routine analysis without additional screening of BLF had been performed, BLF would have been under-reported in 9 cases only.

BLF concentrations ranged from 0.08 to 102.00 µg/mL (median = 0.28 µg/mL ± 22.2 SD) with two potential overdose cases being observed. Misuse of BLF with GHB was seen in one case only. Five cases positive for BLF had a known history of alcoholism, but only two cases had an ethanol concentration of ≥ 50 mg%. BLF was present in combination with PGL in eight cases, and GBP in four cases; PGL or GBP abuse was not indicated in any of those cases.

Discussion

Conclusions

Although other studies have highlighted that BLF is being used recreationally, the results from the study described here identified only one likely case of potential recreational use of BLF. The prevalence of BLF in PM blood was found to be low, suggesting that although BLF abuse may be occurring, misuse-related deaths are not occurring or at least not in the areas covered by this study. This study highlights that it is not cost or time effective for all forensic laboratories to screen for BLF in all cases (at least in this study area). In the future if BLF becomes licensed to treat alcoholism this study will provide useful ‘before licensing’ prevalence data for BLF in deaths. As PGL and GBP have become controlled Class C drugs in the UK from April 2019, there may be a potential shift in drug use from PGL and GBP abuse to BLF in the near future.
Abstract ID 354
Oxycodone findings and CYP2D6 function in post mortem cases.

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Aim
In Sweden, oxycodone is a widely prescribed drug, which is reflected by the increasing number of oxycodone findings in post mortem cases. Between 2012 and 2018 the number of oxycodone findings in post mortem cases doubled, from 2% to 4%. Oxycodone metabolism is mediated partly by the enzyme Cytochrome P450 2D6 (CYP2D6). The gene that encodes for CYP2D6 is polymorphic which means that the concentration of oxycodone in blood may be influenced by the metabolic capacity on individual level. In literature, four groups within the Caucasian population are defined depending on the metabolic capacity. The frequencies are as follows, poor metabolizers (PM) 5-10%, intermediate metabolizers (IM) 10-40%, extensive metabolizer (EM) 50-80%, and ultra-rapid metabolizers (UM) 2-10%. Therefore, we aimed at investigating if oxycodone concentration and genetic variants in CYP2D6 differ in frequency between four categories, divided by cause of death (CoD): 1) intoxication cases where oxycodone was considered the primary CoD, 2) intoxication cases where oxycodone was considered to contribute to death, 3) intoxication cases where oxycodone did not contribute, and 4) other CoD.

Methods
Post mortem whole blood were screened for oxycodone with an LC-QTOF method. Depending on the oxycodone concentration from the semiquantitative screening analysis, either an LC-MS/MS method or a GC-NPD method was used to confirm and quantitate oxycodone. Cases positive (>5 ng/g) for oxycodone in femoral blood were included in the study if at least 0.5 gram of blood was available for DNA extraction. The cases were consecutively collected during the second half of 2015 and during entire 2018. DNA was extracted from 200 µL whole blood by incubation with proteasas K, followed by extraction with an Arrow robot by using Blood DNA 500 kit (Qiagen). CYP2D6 genotype were determined using pyrosequencing for the variants *3, *4 and *6, and digital droplet PCR for copy number variation. Based on the number of functional and non-functional alleles determined, the cases were divided into either PM, IM, EM or UM. Statistical analyses were performed using GraphPad Prism 8.0.1 to determine the relationship between CoD, oxycodone concentration and CYP2D6 genotype. The non-parametric Kruskal-Wallis was used when comparing oxycodone concentrations between groups, and χ2-test when comparing categorical data between groups. A p-value <0.05 were considered significant.

Results
In total, 304 cases representing entire Sweden were positive for oxycodone in femoral blood during the collection period. In 12 cases CoD was undetermined and in 13 cases there was not enough sample to perform DNA extraction. Therefore these were excluded from further analysis. The study population included 279 cases, 105 from 2015 and 174 from 2018. Gender distribution were 38% women and 62% males and the median age was 55 years. The cases were categorized into four groups based on the CoD previously determined by forensic pathologist. The distribution of cases in each group and oxycodone concentration (median, range in µg/g) were as follows: 1) 29 (0.50, 0.10-11.3) intoxication cases where oxycodone was considered the primary CoD, 2) 84 (0.20, 0.006-17) intoxication cases where oxycodone was considered to contribute to death, 3) 39 (0.060, 0.005-0.4) intoxication cases where oxycodone did not contribute, and 4) 127 (0.044, 0.005-7.1) other CoD. The CYP2D6 frequencies and oxycodone concentrations (median, range in µg/g) were: PM 8% (0.10, 0.060-17), IM 36% (0.10, 0.005-1.6), EM 54% (0.060, 0.005-17) and UM 2% (0.15, 0.005-0.60). There were no significant differences in concentration of oxycodone between CYP2D6 genotype. However, there were significant differences in oxycodone concentration between all CoD-groups except 3 and 4. No significant differences were found when comparing CYP2D6 genotype distribution between the four CoD-groups, suicide cases included or excluded.

Discussion
In Sweden, a patient’s metabolic capacity is not routinely determined prior to prescription of oxycodone and therefore an overrepresentation of poor metabolizers could explain accidental deaths when oxycodone is introduced. However, this was not confirmed in our population. Nor did we find a correlation between the metabolic capacity and femoral blood concentrations of oxycodone. An explanation for this can be that the doses administered and the time since last intake was unknown. For opioids, tolerance to the drug effects are common and result in wide and overlapping concentration ranges between living and deceased. However, in our population we did find a significant difference between oxycodone deaths, deaths where oxycodone contributed, and other CoD. Based on our data, concentrations above 0.5 µg/g femoral blood are likely to have contributed to death whereas concentrations below 0.1 µg/g are unlikely to have contributed to acute toxicity.

Conclusions
Examining oxycodone concentrations alone revealed no impact from metabolic capacity of the CYP2D6 enzyme. However, significant differences in oxycodone concentrations were seen between different CoD and a potentially toxic level is suggested to 0.5 µg/g in femoral blood. Future prospects include evaluation of the contribution to the toxicity by oxymorphone produced by the CYP2D6 enzyme.
Abstract ID 361
A UHPLC-MS/MS method for the detection of drugs and pharmaceuticals in human bone.
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Aim
In some forensic cases, where the body has undergone significant decomposition, skeletonization or fragmentation, the analysis of bone samples may provide the only source of toxicological information. For this reason, the necessity for the development of an appropriate method for the detection of drugs and pharmaceuticals in human bones is crucial.

The scope of this work was the development of a sensitive, specific but also robust analytical tool that would enable the analyst to provide solid data from “difficult” samples and quantify trace amounts of drugs. Due to the nature of the samples and the “gravity” of the anticipated result, detection and identification of drugs should be impeccable. Finally analysis should not be lengthy and should not cause system degradation such as column or source capillary blockage. It can be understood that meeting such requirements necessitates the optimization of the LC–MS analysis in both ends: LC and MS.

Methods
A UPLC-MS/MS method was developed, on a reversed-phase column (C18) and a gradient of MeOH over H2O with a total analysis time of 17 minutes for 27 drugs and pharmaceuticals. MRM mode was applied for the detection and quantification of all 27 analytes of interest by monitoring selected transitions. The target compounds comprise opiates, cocaine, cannabinoids, amphetamines, benzodiazepines, antipsychotics and antidepressants. Sample pretreatment process was also studied.

Results
The best results of the sample pretreatment study were obtained after washing for the removal of external bone contaminants, drying and grinding. Extraction of the analytes of interest was accomplished by adding 3 mL of methanol and 12.5 μL of NH4OH (13.4 M) to adjust the pH at 10 in 1 gr of powdered bone, stirring for 5 hours, ultrasonication, filtration, evaporation and reconstitution with 300 L of mobile phase.

The validation of the method performed on spiked bone samples for selectivity, carry-over, limit of detection (LOD), limit of quantification (LOQ), recovery, matrix effect, accuracy and precision (RSD%). The proposed method was applied to the analysis of real bones and bone marrow of chronic drug abusers collected post mortem and after one-year burial, revealing the presence of drugs which were not previously detected in conventional samples such as postmortem blood.

Discussion
LC–MS/MS analysis of bone offers potential for the unambiguous detection and quantitative analysis for different kind of pharmaceuticals and drugs in low concentration. The optimization of the pretreatment method leads to the development of an efficient and quick method (about 6 h in total duration) which provides an additional benefit in comparison to reports from the literature, where typically samples are left overnight for extraction. The method was validated extensively and was subsequently applied in the analysis of samples from seven forensic cases. In these samples, 36 drugs were successful identified; of these 30 could be quantified. An important finding is the fact that 26 compounds were detected and 20 were quantified in bone and bone marrow one year after burial. These results provide evidence to support bone analysis as a useful tool of forensic investigation, especially in cases where other specimens are missing.

Conclusions
The most important conclusions to be exported is that forensic toxicological analysis is not at dead end when common biological samples (blood, urine, tissues or hair) are missing. Even when the only available sample is a part of a skeletonized human body, dedicated methods can provide solid data to the legal medicine expert.
Abstract ID 431
Death involving pong pong seed (Cerbera odollam).
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Aim
Cerbera odollam and Cerbera manghas (commonly known as pong pong, sea pong pong, suicide tree) are both tropical plants found in south Asia, Pacific islands, north Australia and Madagascar. Their fruits, which look similar to mango (hence also known as sea mango), comprise a fibrous husk with an extremely poisonous kernel. The kernel contains cardiotoxic heterosides including cerberin, cerberoside and neriifolin which can cause fatal cardiac glycoside toxicity. Ingestion of as little as one kernel can cause severe toxicity with vomiting, hyperkalemia, bradycardia, heart block, and death. The seeds of C. odollam were reported to be responsible for hundreds of deaths worldwide, with 537 recorded deaths from 1989 to 1999 in the southern Indian state of Kerala alone. In this abstract, two fatal suicide cases involving ingestion of pong pong seeds and the detection of the toxin neriifolin in the biological specimens were presented.

Methods
Samples submitted for toxicology testing were subjected to liquid-liquid extractions under acidic condition with ethyl acetate, and under basic condition with 1-chlorobutane. The extracts were analysed by GC/MS and liquid chromatography with ion-trap MS (Toxtyper by Bruker). MSn spectra and retention times for neriifolin and cerberin were added to the LC-IT/MS library for the screening of these toxins. Quantitation of neriifolin in the blood sample was performed on the basic extract by LC-MS/MS using a 100 mm x 2.1 mm Accucore C18 column, and oleandrin as the internal standard.

Results
The first case occurred in 2017 where the decedent, a 39-year old male, was admitted to the emergency department, conscious but breathless. He told the doctor he had consumed 10 tablets of Panadol and one pong pong seed, believed to have been plucked from a tree near his house. He died about 3 hours later. The postmortem femoral blood specimen was found to contain neriifolin (~33 ng/ml), acetaminophen (49 mcg/ml), amisulpride, citalopram, zolpidem, etomidate, lamotrigine, lignocaine and metoclopramide (all at therapeutic or sub-therapeutic levels). Similar drugs, together with atropine and loperamide were detected in the postmortem urine specimen.

In the second case which occurred this year, the decedent was a 22-year old female who confessed to her psychiatrist during her session that she had taken 2 pong pong seeds bought over the internet the day before. Then she complained of dizziness and nausea, and was quickly referred to the emergency department where an ante-mortem urine specimen was taken. She passed on about 8 hours later. Her postmortem femoral blood specimen was found to contain neriifolin (~50 ng/ml), paroxetine (980 ng/ml), quetiapine (80 ng/ml), amiodarone (6.2 mcg/ml), 7-aminoclonazepam, phenylbutazone and lignocaine (all at therapeutic or sub-therapeutic levels). The toxicology result for the ante-mortem urine specimen revealed similar drugs, with atropine, cetirizine and chlorpheniramine.

Cerberin was screened in both cases but was not detected in the samples.

Discussion
Based on our search in published English articles, there was only a group of cases reported by Maillaud et al. where neriifolin was detected in the blood specimens of patient/deceased, and these were due to ingestion of coconut crabs that fed on the toxic kernels of C. manghas. All other pong pong seed poisoning reported in the literature was often inferred based on clinical presentations, biochemistry tests and/or case histories, and not the positive identification of a causative agent. In our two cases, pong pong seed ingestion was confirmed by the detection of neriifolin in the biological specimens.

Conclusions
Outside its indigenous geographic region, poisoning by C. odollam is seldom encountered but this has changed thanks to the internet. With ease of access of the seeds online and increasing knowledge of it being used for suicide, toxicology laboratories should thus be equipped with the means of detecting such cardiotoxic heterosides.
Abstract ID 498
Relationship between beta-hydroxybutyrate (BHB) concentrations in post-mortem blood and cause of death.

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Aim
Sudden unexpected death caused by diabetic or alcoholic ketoacidosis is easily overlooked due to the non-specific symptoms. A correct diagnosis often depends on whether ketone body analysis is performed in post-mortem body fluids. Although the acid beta-hydroxybutyrate (BHB) is the most abundant ketone formed in the body during fasting, decompensated diabetes mellitus and alcoholism, routine analysis in post-mortem investigations often includes only a neutral ketone, acetone. Since 2010, BHB and acetone have been routinely analyzed in post-mortem blood samples at the Department of Forensic Sciences (DFS) at Oslo University Hospital, Norway. BHB was included to ascertain whether it could provide valuable information in determining the cause of death.

The aim of this work was to investigate the relationship between BHB and acetone concentrations in post-mortem blood and the cause of death, and hence to evaluate the usefulness of BHB analysis in post-mortem cases.

Methods
From our database on forensic autopsy cases routinely examined from January 2012 to December 2015, there were 3520 cases for which the autopsy was performed at DFS and BHB and acetone were analysed in post-mortem blood. BHB and/or acetone were detected in post-mortem blood in 376 cases, and these cases were paired with data from the Norwegian Cause of Death Register. The cases were categorized into three groups according to the main underlying cause of death: “Diabetes-related” (DIA, n=38), “Alcohol-related” (ALC, n=35) and “OTHER” (n=303). The DIA deaths represented diagnoses such as diabetic ketoacidosis and hypoglycaemic/hyperglycaemic coma; ALC deaths represented diagnoses such as alcoholic cirrhosis/gastritis/intoxication/ dependency, and OTHER deaths represented diagnoses such as malign/heart/infectious disease, injuries and drug intoxication. Analysis of BHB in blood was performed using UHPLC-MS/MS with a cut-off of 500 µM (52 mg/L), and analysis of acetone was performed using HS-GC-FID with a cut-off of 1500 µM (87 mg/L). For the purpose of the study, the acetone method was also validated for a cut-off of 400 µM (23 mg/L), in order to evaluate if pathologically elevated BHB concentrations can be reliably predicted by using a more sensitive acetone method.

Results
In DIA deaths, the concentrations of BHB and acetone were significantly higher than in ALC (p<0.001 for both BHB and acetone) and OTHER (p<0.001 for both BHB and acetone) deaths. The median (range) concentration of BHB was 6448 (655–12590) µM in DIA deaths, 2924 (622-14933) µM in ALC deaths and 1087 (0-13467) µM in OTHER deaths. The corresponding concentrations of acetone were 4600 (0-11000), 0 (0-5400), and 0 (0-8000) µM, respectively.

Discussion
There was a strong correlation between the acetone and BHB concentration (Spearman’s rho = 0.56, p<0.001), which was highest in DIA and ALC deaths (Spearman’s rho 0.63 and 0.61, respectively) compared to OTHER deaths (Spearman’s rho 0.32). In 33 deaths (8.8%), the BHB concentration was above the pathologically significant threshold of 2400 µM (250 mg/L), yet there was no acetone detected above 1500 µM. In seven deaths (1.9%), the BHB concentration was above 2400 µM, yet there was no acetone detected above 400 µM. One of the latter cases was diagnosed as diabetic ketoacidosis in the Cause of Death Register.

Conclusions
Our findings indicate that BHB is a more reliable marker of ketoacidosis than acetone, even if a sensitive method for acetone detection is applied, and that analysis of BHB should be routinely performed in post-mortem investigations as the BHB blood concentration also directly indicates the acid-base balance in the body.
Abstract ID 534
Development and validation of a method for quantification of 28 psychotropic drugs in post mortem blood samples by modified micro-QuEChERS and LC-MS/MS.

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Aim
In the last decade, a huge increase of antidepressants use (and abuse) has been reported worldwide. Even the new generation of these drugs have been associated with suicidal and accidental deaths, consequently, these drugs are often encountered in clinical and forensic cases. The development of new sample preparation alternatives in analytical toxicology, leading to quick, effective, automated and environmentally friendly procedures is growing in importance. One of these alternatives is the QuEChERS, also known as dispersive SPE extraction. This approach, which was originally developed for the analysis of pesticide multi-residues, produces cleaner extracts than the LLE, besides facilitating the separation of aqueous and organic phases. Especially in analytical toxicology, QuEChERS sample preparation has shown a fast, reproducible and effectively approach. Despite this, there are few published studies on the miniaturization of this technique for the forensic toxicology area, especially in post mortem analyzes. The aim of this work was to develop and validate an analytical method based on a modified micro-QuEChERS and liquid chromatography tandem mass spectrometry (LC-MS/MS) to the determination of 28 psychotropic drugs in post mortem blood samples.

Methods
For sample preparation, 300 µL of acetonitrile was transferred to a polypropylene tube containing 100 mg of QuEChERS salt (Q150 AOAC method, Restek®), followed by 200 µL of ultrapure water, 100 µL of blank post mortem blood (negative samples previous analyzed by laboratory routine screening procedure) and 10 µL of internal standard (mix of deuterated antidepressants at 1 µg/mL in methanol). The tube was capped, homogenized at 7 m/s for 20 s (3 cycles) and centrifuged at 10,000 rpm/10 min. The organic phase (100 µL) was diluted in the mobile phase A (100 µL) and transferred to LC vials, and 2 µL injected on LCMS8060 (Shimadzu®, Japan). Separation was performed in biphenyl column (100 x 2.1mm, 2.7µm, Restek®), with mobile phase composed by (A) water and (B) methanol, both containing 0.1% formic acid and 2 mmol/L ammonium formate (gradient elution). The flow rate was set to 0.4 mL/min, with 8.0 min run time. The mass spectrometer was equipped with an electrospray ionization source (ESI), operated in positive mode. The source parameters optimized were: heat block temperature 400 °C; ion spray voltage 4.0 kV; nebulizer gas (N2) flow 3 L/min; desolvation line temperature 250 °C; drying gas (N2) flow 10 L/min; heating gas (N2) flow 10 L/min; and collision induced dissociation gas pressure (Ar) 270 kPa. The analyses were performed in multiple reaction monitoring (MRM) mode. For each compound, two MRM transitions were selected, one for quantification and one qualifier for confirmation identification using the MRM ratio as identification criteria (20% maximum tolerance window).

Results
The developed method was able to analyze 28 substances in 8.5 minutes of chromatographic run. Limits of detection (LOD) and quantitation (LOQ) were 1 ng/mL. No endogenous (analyzes of 10 different post mortem blood samples) or exogenous interference were observed. The linearity was evaluated from 1 to 500 ng/mL, and shown good performance (r > 0.99, 1/x2). Matrix effect were evaluated for all analytes, and ranged from -10.7 to +37.3% Method precision (ANOVA, greater than 18.5%) and bias (greater than 18.9%) were evaluated in three concentrations levels. Bias was also evaluated by analysis of certified controls plasma samples (Chromsystems®). Until now, the developed method was applied to the analysis of 40 real samples, being citalopram the most commonly found (12 positive samples, with range between 1.0 – 16.7 ng/mL).

Discussion
For the linearity, the test F was applied to evaluate if the variance between the replicates remained constant for the different levels of the calibration curve. Thus, six weighting factors were analyzed: 1/x, 1/x², 1/x0.5, 1/y, 1/y², 1/y0.5. For each one of the six conditions, were calculated the correlation coefficient (r) and sum of residual regression errors (ΣRE%). Then, it was possible to choose the weighting factor that had the lowest ΣRE%. The norfluroxetine and norsertraline, the method didn't achieve the acceptance criteria of identification at the LOQ and, therefore, were classified as semi-quantitative. Two calibrations curves were extracted at the same day, in blank plasma and the other in blank post mortem blood, for the evaluation of certified controls samples and shown good correlation. According to SWGTOX, the acceptance criteria for matrix effect was ±25%, only two analytes presented concentration higher than 25%: olanzapine (+37.7%, high QC) and levopromazine (+28.6% and +27.5% at low and high QC, respectively). Despite the significant matrix effect, the method sensitivity (LOD/LOQ), precision and bias were not affected.

Conclusions
A sensitive method based on micro-QuEChERS and LC-MS/MS was developed to quantify 17 antidepressants, 7 antipsychotics and 2 metabolites, and semi-quantify 2 metabolites in post mortem blood samples. The method requires low sample volume (100 µL) and combine simple sample preparation and fast instrumental analysis (shorter than 8.5 min).
Abstract ID 11  
Concentrations of Amphetamine and Methamphetamine in Whole Blood Samples Obtained In Drugs and Driving Cases (OUI) in the Commonwealth of Massachusetts.  
Jeffery Hackett  
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¹Private,  
²Massachusetts State Police Crime Laboratory  

Aim  
In this presentation, attendees will learn about the extraction and analysis method for Amphetamine (AMP) and Methamphetamine (MAMP) from the whole blood samples in drug/driving cases employing commercial available solid phase extraction (SPE) cartridges and analysis of the extracts using Liquid Chromatography-Mass Spectrometry (LC-MS/MS).  

At therapeutic doses, AMP/MAMP cause emotional and cognitive effects such as euphoria, change in desire for sex, increased wakefulness, and improved cognitive control. These compounds may induce physical effects such as decreased reaction time, fatigue resistance, and increased muscle strength. In the case of operating motor vehicles even therapeutic doses may impair the ability of the driver to control the vehicle safely. The information presented will allow analysts to better interpret the concentrations of AMP/MAMP in whole blood samples.  

Methods  
In this study, whole blood samples taken from motor vehicle operators were submitted to the laboratory in 10 mL grey top tubes (Sodium Fluoride/Oxalate preservative). In each case, two samples of whole blood were submitted as part of the "Operating Under the Influence" (OUI) (also known as Driving Under the Influence of Drugs (DUID) kit in other US states). The motor vehicle operators were apprehended by police officers mainly due suspicion of impairment and after standard field sobriety testing with and without a collision occurring. From submitted cases, 1.0 mL aliquots of whole blood (calibrators, controls, and test samples each containing deuterated internal standards) were diluted with 3.0 mL of aqueous phosphate buffer (0.1 mol/L, pH 6) and vortex mixed and centrifuged for 10 minutes at 3000 rpm. Each test sample was analyzed in duplicate. The supernatant liquid was applied to mixed mode ion exchange (C8/SCX) SPE column that had previously been conditioned with: methanol, deionized water (DI H2O) and phosphate buffer (3 mL, respectively). Each SPE column was washed with DI H2O, aqueous acetic acid, methanol (3 mL, respectively) and dried for 10 minutes under full flow using a positive pressure solid phase extraction manifold and nitrogen as the drying gas. Each SPE column was eluated with 1 x 3 mL of a solution consisting of methylene chloride-isopropanol-ammonium hydroxide (78-20-2) and the eluates were collected in glass tubes. To the eluate was added 100 µL of LC-MS/MS mobile phase, vortex mixed and were evaporated to approximately 100 µL under a gentle stream of nitrogen at 35 °C, to this solution 100 µL of LC-MS/MS mobile phase was added and vortex mixed before being transferred to individual auto sampler vials for analysis by LC-MS/MS. LC-MS/MS was performed using a gradient program with a mobile phase consisting of acetonitrile containing 0.1% formic acid/aqueous 0.1% formic acid. Tandem mass spectrometry was carried out in positive multiple reaction mode (MRM) details presented. Liquid chromatography was performed using polyaromatic phase column (50mm x 2.1 mm (5µm)) at a flowrate of 0.5 mL/minute.  

Results  
The limits of detection/ quantification for this method for AMP/MAMP were determined to be 5.0 ng/mL and 10 ng/mL, respectively for the analytes. The AMP/MAMP method was found to be linear from 10 ng/mL to 1000 ng/mL (r²>0.999). The AMP/MAMP recoveries were found to be greater than 95%. Interday and Intraday variation of method were found to < 7% and < 9 %, respectively. Matrix effects were determined to be < 5% for AMP/MAMP SPE method. Details regarding the concentrations of AMP/MAMP found in genuine drugs and driving cases ranged from: 174 Males: median age: 28 years old (AMP: 15ng/ mL to 506 ng/ mL: median=378ng/ mL), (MAMP: 34 ng/ mL to 882 ng/ mL: median= 620ng/ mL); 82 Females: median age: 24 years old (AMP:12 ng/ mL to 472 ng/ mL: median= 288ng/ mL), (MAMP: 28ng/ mL to 724ng/mL: median = 545ng/ mL).  

Discussion  
This data compares the incidence and concentrations of AMP/MAMP found in whole blood samples of drivers suspected of driving under the influence of drugs including AMP/MAMP. These types of samples are frequently presented to forensic toxicology laboratories for analysis and interpretation. The mean concentrations of AMP/MAMP in the blood of male drivers are higher than female (AMP:MAMP: median age: 28 years old (AMP: 12 ng/ mL to 472 ng/ mL: median= 288ng/ mL), (MAMP: 28ng/ mL to 724ng/mL: median = 545ng/ mL). The mean age of these operators of motor vehicles are similar (28 for males, and 24 for females). The data presented relates only to AMP/MAMP, other drugs may be present.  

Conclusions  
The data shows that AMP is a more frequently observed drug than MAMP in blood samples taken in the Commonwealth of Massachusetts obtained in drugs and driving cases. It must be noted that AMP is a metabolite of MAMP and both compounds are psychoactive which adds to the effect upon impairment of the drivers. This method of extraction by SPE offers analysts an efficient procedure, by using the mobile phase as a keeper solvent eliminates loss of analytes during evaporation. LC-MS/MS reduces analysis time by eliminating the need to derivatization as is needed in GC-MS methods.
Abstract ID 93
The rise of flubromazolam as counterfeit Xanax®: detecting and managing the emergence of an NPS.

Marie-Jo LaJOie

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Aim
By definition, novel psychoactive substances (NPS) appear without warning in forensic toxicology laboratories’ cases. While the detection of a NPS is sometimes an isolated event, it can also be the harbinger of an increased presence on the covered territory.

In this work, we describe how the appearance of the designer benzodiazepine flubromazolam in the Province of Quebec (Canada) was detected by the forensic toxicology laboratory, and how the analytical workflow was amended with the aim of successfully detecting its presence in cases.

Additionally, data is presented for all 11 cases where flubromazolam was detected up to February 2019.

Methods
The Laboratoire de sciences judiciaires et de médecine légale performs all forensic toxicology analyses required for postmortem (coroner) cases, sexual assault and driving under the influence cases for the Province of Quebec (Canada).

Up until December 2018, almost all driving under the influence of drugs (DUID) cases treated were carried out under the drug evaluation and classification program (DECP), where an individual arrested for DUID is submitted to a 12-steps physical evaluation and interviewed by a drug recognition expert (DRE) officer, followed by collection of a bodily fluid sample, mostly urine.

Targeted screening and confirmation is achieved by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) following a protein precipitation extraction. This method, validated according to SWGTOX, ISO 17025:2005 and CAN-P-1578 standards, performs quantification of 60 xenobiotics and decision point qualitative determination of 40 additional drugs and metabolites.

Following this analysis, a general unknown screening (GUS) by gas chromatography coupled to mass spectrometry (GC-MS) is carried out if further investigation is deemed necessary, for example if there are no toxicological findings in a DUID case. In this case, samples are extracted by solid phase extraction using a Waters Oasis® HLB cartridge.

Results
The first instance of flubromazolam detection occurred in October 2017. In a DUID case, the suspect stated he was prescribed Xanax®, but analyses found no alprazolam or metabolite in the urine sample collected. This incoherence was further investigated by carrying out a general unknown screening by GC-MS, where flubromazolam was identified. Following this case, declaration of Xanax® consumption by the suspect to the DRE officer or possession of pills visually identified as Xanax® prompted a GC-MS GUS analysis. In total, flubromazolam was detected in 11 DUID cases between October 2017 and February 2019. The changes instigated in the analytical workflow were responsible for the identification of 7 of these cases, with three drivers intercepted with counterfeit Xanax® tablets and four others declaring Xanax® use. In the remaining four cases, flubromazolam was discovered fortuitously following a GUS analysis instigated because the reported signs and symptoms or DRE officer determination did not fit the findings of the LC-MS/MS targeted screening and confirmation.

In a majority of cases (n = 9), the drivers were 18 to 29-year-old males; in the remaining two cases, a 20-year-old female and 52-year-old male were intercepted. Polydrug use was highlighted in all cases but one, most frequently with cannabis (metabolite, 82% of cases), cocaine (45%) and methamphetamine (27%). In all cases, DRE officers reported slurred speech, slowed reaction time and drowsiness. Several drivers displayed horizontal and/or vertical nystagmus (82%) or a lack of balance (45%). Severe confusion was also noted in two cases. However, these symptoms must be interpreted with care in the light of the evidenced polydrug consumption.

Discussion
Frequently reported effects for the designer benzodiazepine flubromazolam are similar to most benzodiazepines, including drowsiness and motor incoordination. It is therefore not surprising to find it sold as Xanax® tablets on the illicit market. Nevertheless, users are unaware of this substitution, which can lead to several unanticipated adverse effects. This rise in flubromazolam cases in the Province of Quebec (Canada) points to several important elements in detecting and managing the emergence of a NPS in a given territory. First, details about the case and its circumstances are important in order for the forensic toxicologist to perform an optimal analysis, and a black box approach to forensic toxicology is likely to miss such phenomenon. Second, the forensic toxicologist must remain alert to the signs of possible NPS involvement in cases and expand analytical testing in such situations.

Conclusions
Flubromazolam was first identified in a DUID case in October 2017, which prompted modifications to the analytical workflow. However, 4 out of the 11 cases detected since then were fortuitous discoveries, demonstrating the low efficiency of the implemented approach. Considering these results, flubromazolam has now been integrated to the standard targeted screening and confirmation procedure. A multiple fold increase in detection of flubromazolam is expected as a result of this change, deemed necessary not only for judiciary but also for public health reasons. The sequence of events described here also highlights the necessity of an open, flexible and reactive analytical method where prevalent NPS can be promptly added.
Abstract ID 124
Katerina Liveri
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Aim
The Department of Forensic Chemistry and Toxicology of the Cyprus State General Laboratory is the authorized government laboratory responsible for the analysis of seized materials and biological samples suspected for drugs of abuse, pharmaceuticals and other organic compounds. National drug policies are being evaluated through ongoing research programs and by monitoring indicators such as Narcotest legislation, Drug Related Deaths (DRD) and data collected over the years from road accidents (Human performance toxicology). Chemometric evaluation of the Narcotest data was also performed for 2018.

Methods
To implement the Narcotest legislation, a rapid and sensitive LC-MS/MS method was developed and validated in oral fluid, for the detection of 20 drugs of abuse, medicinal drugs and their metabolites that are under scheduled in the National Drug Law. Analytes were: Cannabidiol, Δ 9-Tetrahydrocannabinol (THC) and the main metabolite 11-nor-carboxy-THC (THC-COOH), cocaine and the metabolites benzoylecgonine, ecgonine methylester, amphetamine-like stimulants: amphetamine, metamphetamine, 3,4- methylenedioxymethamphetamine (MDMA), 3,4- methylenedioxyamphetamine (MDA), 3,4- methylenedioxymethamphetamine (MDEA), pseudoephedrine, ephedrine, the opiates: morphine, codeine and the heroin metabolite 6-acetylmorphine (6MAM), the opioids: oxycodone, dihydrocodeine, buprenorphine and fentanyl. The analysis of post mortem samples and samples collected from road accidents was mainly performed on GC-MS.

For the oral fluid samples, analytes were isolated using liquid – liquid (L/L) extraction and for the blood and urine samples solid phase extraction (SPE) was used mostly in the past.

Results
A total of 366 oral fluid samples analyzed in 2018, after a positive indication at onsite testing performed by the police. Data was also collected over the years from the analysis of post mortem samples, road accidents and seized materials. The evaluation of all the above data showed the need for continuous monitoring of these indicators.

Discussion
The police perform onsite preliminary tests to drugged suspected drivers. If positive, a second OF sample is obtained for the confirmatory test. The Narcotest legislation is being implemented for “zero tolerance” policy. According to the law, a driver is penalized regardless of the level of the drugs detected. “Zero” is specified as the LOD of the targeted analytes.

Statistical analysis of the results for 2018, showed that the substance mostly used was cannabis followed by the combination of cannabis and cocaine. Polydrug use was also observed. Drivers aged between 17 to 55 years old and were almost all male drivers. The laboratory is proceeding in further method development of the detection of new psychoactive substances (NPS) in oral fluid, blood and urine using LC-MS/MS.

Conclusions
The authors emphasize the importance of the collaboration between the Cyprus State General Laboratory, the Police, The National Addiction Authority and other stake holders is essential in order to understand the prevalence of drug use and to develop effective national drug policies and put forward in the European future drug policies.
Abstract ID  148
The Presentation of Hair Strand Analysis Evidence in Court.
Alexander Forrest

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Aim
The aim of this presentation is to stimulate a constructive discussion about the way in which toxicological hair stand analysis results are reported and presented in Court.

Methods

Results

Discussion
For many years the results of hair strand analyses have been reported simply by reference to cut-off values. There are many reasons for this, including the variability of hair as a matrix both between and within individuals, the effect of cosmetic and hygienic treatments, the effect of activities of every day living, for example swimming, and the very wide inter laboratory variation in quality assurance/proficiency testing. An additional, and critical, component in the interpretation of results is the difficulty inherent in the differentiation of passive exposure to drugs from active use on the basis of hair analyses. The Society of Hair Testing proficiency testing exercises in 2018 found the between laboratory C.V varied from 22% For tramadol to 90% for THC-COOH.

The advantage of using cut-off concentrations to report results is that they can be formulated to take account of the variabilities set out above to present the commissioners of the test with an apparently unequivocal result. This approach is not always in the interests of justice and can cause confusion when different laboratories, instructed by different parties in a case, report apparently inconsistent results. In the English Courts this issue was epitomised by the case of re H (H (A Child : Hair Strand Testing) [2017] EWFC 64 (http://www.bailii.org/ew/cases/EWFC/HCJ/2017/64). It is noteworthy that in this case there was no discussion in the reported judgement of the possible effect of cosmetic treatment of the mother’s hair.

The increasing availability of assays that can demonstrate the presence of drug metabolites in hair that can only be found in body fluid and keratinous matrices and not in the environment assists in the differentiation of passive exposure to the drugs of interest from active use. This can be problematic; for example, cocaethylene in hair can be a marker indicating the donor of the sample has used cocaine together with alcohol. However, cocaethylene can be found in illicit and pharmaceutical cocaine and is formed when dissolved in spirits such as high proof rum used to smuggle cocaine across international borders. It can only be a matter of time before a defence is presented on the basis that a drug metabolite in hair is present because the hair has been washed with the urine of a partner or carer who is a drug user.

We would suggest a more nuanced approach to the presentation of hair strand analyses in Court should replace simply reporting that a result is above or below a cut off value, perhaps with a comment to the effect that the in the experience of the laboratory the result reflect occasional, moderate or heavy use of the drug in question. A reasoned interpretation of the result taking into account known potentially confounding factors including hair colour, donor age, gender, cosmetic treatments, life style and declared prescribed and illicit drug use should be presented to the Court, always remembering that the Court and not the toxicologist is final arbiter. This does require the commissioner of the analysis to provide the toxicologist with an appropriate history including the circumstances leading to the commissioning of the examination and to accept that additional analyses may be required over and above those initially requested to answer the questions that the Court has to address. This approach will require the toxicologist to be acutely aware of the problem of cognitive bias, as should every expert who provides opinion evidence to the Court.

Conclusions
Toxicologists reporting hair strand analysis results should move from simply providing analytical results with cut-offs to assisting the Courts as experts by providing evidence based opinions.

Keywords: Hair strand analysis, Cut-offs, Interpretative Reports, Judicial Proceedings
Abstract ID 235
Large-scale study to evaluate the variability in elimination rate of the direct alcohol marker phosphatidylethanol during one month of abstinence.

Katleen Van Uytfanghe
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UGent, FFW, Laboratory for Toxicology

Aim
Phosphatidylethanol (PEth) is a group of phospholipids that is formed in erythrocyte cell membranes in the presence of ethanol only. This makes them a highly specific direct alcohol biomarker. In comparison to other direct and indirect alcohol biomarkers, PEth is promising as its specificity and sensitivity are higher. The aim of this study was to get more insight in the variability of the elimination rate of PEth during a month of abstinence and to evaluate our current limits for PEth for so called social drinkers.

Methods
Over 700 volunteers participating to Tournée Minérale were recruited. Tournée Minérale is an initiative from the Belgian Foundation Against Cancer and the “DrugLijn” Belgium. People participating to this event voluntarily stop drinking for one month. During this period they took three fingerprick blood samples (early, mid and end of February) via self-sampling at home using a volumetric absorptive microsampling device (Mitra®, Neoteryx). These devices allow to collect a fixed volume of blood (in this case 10 µl), irrespective of the hematocrit. Volunteers were instructed remotely (via a leaflet and a video on a dedicated website) on how to self-sample correctly. Self-sampling kits were sent from and back to the lab via regular mail. To be able to interpret the PEth values according to the participants’ drinking habits, a short questionnaire (based on the alcohol use disorders identification test (AUDIT)) was sent to the volunteers along with the sampling kit. PEth 16:0/18:1 was quantified using a fully validated liquid chromatography – tandem mass spectrometry method.

Results
About 650 volunteers completed the study. Based on preliminary data analysis (n = 504) we can see that the majority of the volunteers were female (62.5%), European (99%) and with an even spread in the age range 18 to 60 years. The mean alcohol use ranged from none to over 50 units per week in January, 36% of participants reporting 1-5 units per week. The majority reported drinking two or three times a week (40%), with a frequency of one or two drinks per day (44%). Finally, 36% of the volunteers mostly reported binge drinking (more than 6 units in succession) less than once a month. PEth values ranged (as measured in samples from 240 volunteers so far) from 0.9 to 1142 ng/mL, 0 to 404 ng/mL and 0 to 189 ng/mL at early, mid and end of February respectively. PEth decreased to 0.33 (SD 0.13) times the starting level in mid February and to 0.12 (SD 0.08) times the starting level at the end of February, with no correlation between the ratio of the PEth concentration to the initial PEth concentration (R2 < 0.013 for both time points). The correlation between the average units a week and the PEth concentration can be described by a linear regression line (PEth = 6.4 average units a week + 5.7, r² = 0.51).

Discussion
With 504 questionnaires evaluated and samples from 240 volunteers measured so far, the data analysis -which is still ongoing but will be completed by June 2019- is still preliminary and biased by possible outliers. We succeeded to collect samples from a variety of different classes of social drinkers: from almost abstainers to possible heavy social drinkers. The range in PEth concentration is broad and there is a correlation with the reported average PEth intake in January.

Conclusions
A unique study was set up, in which an unprecedented number of alcohol drinkers was followed up during one month of abstinence, made possible by combining the linkage with the “Tournée Minérale” initiative with microsampling at home. Determination of PEth 16:0/18:1 concentrations in the resulting microsamples revealed that the decrease in PEth is not related to the initial PEth level, with an average 3-fold reduction per 14 days. Also, the decrease in PEth indicates a half-life which is longer than currently reported in literature (3-5 days would result in a ratio after 14 days of abstinence between 0.03 and 0.125). Further analysis is ongoing and the overall results of this study will be reported.
Abstract ID 293
Prevalence of synthetic cannabinoid use among persons undergoing drug testing for cannabis – the impact of new NPS legislation in Germany.
Michaela J. Sommer
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Aim
Synthetic cannabinoids (SCs) represent the largest group of new psychoactive substances and they are often perceived as a legal alternative to cannabis. To prevent the emergence and spread of further chemical variations of SCs, a new German law, the NpSG (act on NPS), took effect on November 26th, 2016. Besides the alleged legality, a primary motive for the use of SCs seems to be to circumvent drug testing while maintaining the experience of a cannabis-like high. In most cases of abstinence control ordered by court (CC) or for driving license regranting cases (DLRC), SCs are not among the routinely tested drugs. For this reason, little is known about the proportion of participants in abstinence programs using SCs as a ‘non-detectable’ cannabis substitute. The aim of the present study was to assess the incidence of SC consumption and to evaluate the effects of the NpSG among people undergoing abstinence control.

Methods
Anonymized urine samples collected in two German federal states between January and November 2015 and January and November 2018 for abstinence control were analysed for SCs and their main metabolites applying a validated LC-MS/MS method. The samples included had been tested routinely for cannabis but not for SCs.

Results
In 2015, the uptake of SCs could be confirmed in 56 of the 809 analysed urine samples, resulting in an overall prevalence of 7% in the study collective. In 2018, the sample collective comprised a total of 831 urine samples. SC consumption was verified in 23, resulting in a total prevalence of 3%. A comparison of the prevalence of both federal states showed a higher prevalence in Northern Bavaria (13% in 2015, 8% in 2018) than in Baden-Württemberg (4% in 2015, 1% in 2018) in both years. In 2015, all positive samples were obtained in the context of CC, whereas all samples from DLRC were tested negative. In 2018, there was one DLRC participant with proven SC consumption. In 2015, metabolites of at least 13 different SCs were detected, with MDMB-CHMICA, AB FUBINACA/FUB-AMB and AB-CHMINACA being the three most prevalent substances. In 2018, metabolites of only 7 different SCs were detected. In 84% of the positive samples at least one synthetic cannabinoid was detected that is not subject to the NpSG, namely cumyl-PEGACLONE (36%), 5F-cumyl-PEGACLONE (52%) and 5F-MDMB-P7AICA (16%).

Discussion
The results show that both the overall prevalence and the prevalence of the two federal states in 2018 declined significantly compared to 2015. The variety of SCs consumed also notably decreased. In both years, however, there was a clear regional difference in prevalence. The results also show that the use of SCs in DLRCs is more of a rarity, but a rather frequent phenomenon among CCs. Most consumers seem to use synthetic cannabinoids as a substitute for cannabis.

Conclusions
The study data show that the prevalence of consumption of SCs by persons undergoing abstinence control programs decreased in all respects, but with remaining regional disparities. Besides other influences like enforcement of legal regulations or popularity, it can be assumed that the NpSG itself also had a relevant impact on the prevalence of SC use among the described populations. As the main part on the persisting ‘THC substituters’ use SCs not regulated by law, it remains important to update legal regulations regularly and to keep analytical methods continuously up-to-date.
Abstract ID 305
Sales of CBD Containing Hemp Products, a New Trend in France.

Jérémy Lelong
Jérémy Lelong, Ysé Jagailloux, Bertrand Brunet
chu poitiers

Aim
Since a few months, specialist stores or so-called “coffee shops” have opened in France, offering enriched products with cannabidiol (CBD). Given a legal grey zone on the sale of these products, it is important to be able to verify the composition to ensure that these products contain just CBD and no tetrahydrocannabinol (THC). Indeed, in France, the THC is a controlled substance due to the psychotropic effects it induces. We show that CBD products may contain THC and for certain products, in a concentration that is not negligible.

Methods
Quantitative analyses for THC and CBD were carried out by liquid or gas chromatography coupled with mass spectrometry (LC-MS/MS or GC/MS). The standard addition method was used to calculate the THC and CBD concentrations after liquid/liquid extraction with heptane/ethyl acetate or by dilution with ethyl acetate. The seized products analyzed were: hemp flowers, coffee pods, hemp oil, honey, food supplements, tablets, ointments and creams.

Results

The THC and CBD concentrations are shown in the table below.

<table>
<thead>
<tr>
<th></th>
<th>CBD</th>
<th>THC</th>
<th>CBD</th>
<th>THC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowers “Black Banana”</td>
<td>9.0 mg/g</td>
<td>1.2 mg/g</td>
<td>Food supplement</td>
<td>23.9 mg/caps</td>
</tr>
<tr>
<td>Flowers “Gorilla”</td>
<td>8.1 mg/g</td>
<td>0.8 mg/g</td>
<td>Tablets “arthrocann”</td>
<td>-</td>
</tr>
<tr>
<td>Flowers “Little µg/g Charlotte”</td>
<td>71.0 mg/g</td>
<td>3.2 mg/g</td>
<td>Ointment “trompetol”</td>
<td>29.1 µg/g</td>
</tr>
<tr>
<td>Coffee with µg/g hemp flowers</td>
<td>119.4 µg/g</td>
<td>3.5 µg/g</td>
<td>Ointment “trompetol”</td>
<td>25.4 µg/g</td>
</tr>
<tr>
<td>Coffee with µg/g hemp seeds</td>
<td>0.8 µg/g</td>
<td>-</td>
<td>Ointment “Dolorcann”</td>
<td>3.2 µg/g</td>
</tr>
<tr>
<td>Hemp oil</td>
<td>27.1 mg/mL</td>
<td>1.7 mg/mL</td>
<td>Cream</td>
<td>1.9 µg/g</td>
</tr>
<tr>
<td>Honey</td>
<td>0.8 µg/g</td>
<td>20.2 µg/g</td>
<td>Cream</td>
<td>2.5 µg/g</td>
</tr>
<tr>
<td>Food µg/g supplement 4%</td>
<td>11.8 mg/caps</td>
<td>46.4 µg/caps</td>
<td>Cream</td>
<td>1.8 µg/g</td>
</tr>
</tbody>
</table>

Discussion
After analysis, a single product contained neither CBD nor THC (Arthrocann) at the limit of quantification of the method (0.5 µg/g). All the other products contained at least CBD at concentrations ranging from 0.8 µg/g to 71 mg/g and for some of them THC at 0.8 µg/g to 3.2 mg/g. The diversity of the products and matrices prompted us to use standard addition technique even if it is a time consuming methodology. We achieved good reproducibility between the aliquots of a same matrix.

Conclusions
In France, it is prohibited to sell THC containing products. With authorization and for some definite usage only, hemp can be grown and used for fibers and seeds if those are containing less than 0.2% THC per gram. Sellers can be prosecuted for drug trafficking, administration of harmful substances, promoting the use of illegal drug, illegal practice of pharmacy. Owners of those “coffee shops” are hiding behind EU laws that are less restrictive than French laws. The future of those stores in France is in the hands of justice.
Abstract ID 355

Drug testing in hair: a powerful tool to approach the epidemiology of polydrug use.

M José Burgueño
M José Burgueño1, Sergio Sánchez2, M Ángeles Castro2, Ramona Mateos-Campos3

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Aim
To establish the epidemiological pattern of drug use in the population subjected to hair testing in the forensic context in a broad area of Spain, with special focus on polydrug use.

Methods
A cross-sectional study on drug use was conducted on 4,232 individuals subjected to drug testing in hair in the forensic context, within a three-year period. Most of the tests were carried out within the framework of criminal cases and the diagnosis of drug addiction in order to mitigate criminal liability. Quantitative test results from GC-MS/EI in selected-ion monitoring mode were interpreted according to the confirmation cut-offs proposed by the Society of Hair Testing (SoHT) to identify chronic drug use. Prevalence of cannabis, cocaine, heroin, ketamine, amphetamine (AP), methamphetamine (MA), 3,4-methylenedioxy-methamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxy-N-ethylamphetamine (MDEA) and methadone chronic uses were obtained, as well as prevalence and patterns of chronic polydrug use. Trends over the period were analysed.

Results
Seventy-nine percent of cases were positive for at least one of the substances analysed: 52% for cannabis, 48% for cocaine, 10% for heroin, 17% for AP, 13% for MDMA, 0.3% for MA, 0.1% for MDA, 0% for MDEA, 0.8% for ketamine, and 11% for methadone. Most of positive results (58%) came from users of two or more psychoactive substances; the most frequent pattern of combined use was cannabis and cocaine use, which accounted for 16% of total positive cases. Polydrug use was the most common practice among users of all the substances analysed, with the lowest proportion in the case of cannabis users (69%) and reaching 100% in ketamine users.

Discussion
Rates of cannabis, cocaine, AP, MDMA and heroin use increased over the three-year period under review, while rate of methadone decreased. Users of ketamine, methamphetamine and MDA together accounted for less than 1.5% of positive cases, with the latter substance being fully testimonial. Consumption of cannabis and cocaine in combination with other drugs increased, while the monoconsumption as well as the combined use of both substances decreased. AP use increased in both mono- and polyconsumption patterns. The polyconsumption of MDMA accounted for more than 96% of the consumption of this substance.

Conclusions
Polydrug use was the most prevalent pattern among the population undergoing hair testing in the forensic context in a broad area of Spain and it increased over the three-year period under review. Use of cocaine and cannabis was the most frequent combination, but it decreased during the period. In contrast, prevalence of patterns comprising three or four drugs increased: cannabis and cocaine plus amphetamine and/or MDMA; cannabis, amphetamine and MDMA; cannabis, cocaine and heroin. These trends represent an undeniable increment in health and psychosocial risks associated with drug use, given that polydrug use enhances the effects and reinforces addiction.
Abstract ID 365
The United Nations Office on Drugs and Crime Early Warning Advisory on New Psychoactive Substances.

Conor Crean
Conor Crean
United Nations Office on Drugs and Crime

Aim
In 2016, the United Nations General Assembly Special Session on the World Drug Problem (UNGASS) decided to prioritise the most harmful, persistent, and prevalent NPS for international action.

Methods
Following this decision, the Commission on Narcotic Drugs (CND) in its resolution 60/4, entitled “Preventing and responding to the adverse health consequences and risks associated with the use of new psychoactive substances”, mandated the development of the UNODC Early Warning Advisory on NPS – Toxicology Portal (Tox-Portal) in order to address the resolution adopted by Member States.

Results
The Tox-Portal is an online tool developed in collaboration with The International Association of Forensic Toxicologists (TIAFT) that collects data on toxicology and harm related to the use of NPS at a global level.
Abstract ID 400
Drug driving per se limits in England and Wales: A review of data from over 9,000 cases.
Matthew Christopher
Matthew Christopher, Emily Evans, Susan Grosse, Elizabeth Spencer, Neville Isles
Eurofins Forensic Services

Aim
In early 2015, England and Wales introduced Section 5A of the Road Traffic Act 1988, legislation making it an offence to drive a motor vehicle with a blood concentration of any specified drug above a specified limit. Eight illicit drugs/metabolites (cocaïne, benzoylecgonine (BZE), delta 9 tetrahydrocannabinol (THC), ketamine, methyamphetamine, MDMA, 6 monoacetylmorphine (6 MAM) and lysergic acid diethylamide (LSD)) and nine medicinal drugs (diazepam, oxazepam, temazepam, clonazepam, lorazepam, fluindazepam, morphine, methadone and amphetamine) were each given specified limits following consultation with an expert panel.

Findings from over 9,000 Section 5A cases processed by our laboratory will be presented along with some of the common defences that we have encountered in those cases that have made it to court.

Methods
Section 5A casework samples are analysed at the Eurofins Forensic Toxicology laboratory, Teddington, UK, by two United Kingdom Accreditation Service (UKAS) accredited methods using GC MS/MS and LC-MS/MS. The laboratory has been collecting casework data since the introduction of Section 5A legislation, including drug concentrations detected and incident to sampling time intervals. The data presented will be a result of the interrogation of this internal casework database.

Results
Of the 9,156 cases reviewed, 6,617 (72%) were found to be above the limit for one or more drugs on the panel. 5,371 (81%) of these cases were above the limit for THC, with the next most prevalent being BZE (cocaïne breakdown product) with 2,310 (35%) followed by cocaine itself with 960 (15%). It is likely that these numbers are skewed upwards due to the fact that cannabis and cocaine are currently the only drugs tested for by roadside oral fluid tests in England and Wales, which give officers the power of arrest should they be detected.

Of the 6,617 cases found to be above a specified limit, only 214 (3%) were over the limit for the 'medicinal' drugs on the panel, dropping to 85 (1%) if amphetamine and morphine were removed (those results having possibly resulted from the illicit use of amphetamine and heroin).

The impact of the time interval between incident and blood sampling (x= 2hrs 18mins) on the likelihood of finding Section 5A drugs in excess of the specified limits will also be discussed, with a particular focus on THC.

A brief summary of the common defences that we have encountered (including passive inhalation of cannabis smoke) will also be presented.
Abstract ID 428
DUID Trends post-Cannabis Legalization and a Comprehensive Analytical Approach for DUID Casework.
Luke N. Rodda
Luke N. Rodda, Alessandra M. Rivera, Megan Farley, Sue Pearring
San Francisco Office of the Chief Medical Examiner

Aim
 Californians voted to approve the Control, Regulate and Tax Adult Use of Marijuana Act (Proposition 64) that then led to the passing of legislation allowing for commencing state-wide recreational sale and use of cannabis in January 2018. Subsequent health, legal and safety implications were largely conceptualized and initiated only after cannabis use was permitted in the most populous state in the country. Additionally, impaired driving from the use of other recreational and therapeutic drugs continues to rise.

This two-part presentation aims to discuss drugs observed in DUID casework within the City and County of San Francisco, and to demonstrate an analytical approach that achieves a comprehensive scope of drug testing in suspected drug-impaired drivers.

Methods
All misdemeanor and felony DUID casework from 2016 to 2018, inclusive, were selected for trend and descriptive statistical analysis, encompassing the first 12-month period where cannabis legalization was contemporary in 2018. Alcohol, cannabis and other drugs cases were divided into alcohol-only (BAC >0.01%), cannabis-only and other drug-only groups, with subsequent combinations of each also grouped when co-detected.

Analytical methodology utilizing LC-MS/MS was fully validated to SWGTOX and international guidelines, and included: Bias (Accuracy) and Precision; Dilution Integrity; Carryover; Interferences, Selectivity, and Crosstalk; Limit of Detection (LOD); Limits of Quantitation; Matrix Effects; Processed Sample Stability; and Linearity experiments.

Results
In total, over 2000 drivers were apprehended for blood collection and analysis over the 3 years within the county. The median age and time of collection following the incident were ~32 years and ~1.7 h, respectively, whilst 80% of the drivers were male. The top five substances detected were alcohol (~70%), delta-9 THC (~30%), methamphetamine (~12%), cocaine (~10%) and alprazolam (~5%). When ethanol and delta-9 THC were co-detected, the average concentrations of both were 0.15% and 3.8 ng/mL, respectively. Whilst when detected separately, the average concentrations of the two substances were 0.18% BAC and 7.8 ng/mL for delta-9 THC, double the concentration when detected in combination with alcohol.

Cannabinoids, hallucinogens, cocaine, amphetamines, opiates and synthetic opioids, benzodiazepines, Z-drugs, sedating antihistamines, sedating anticonvulsants, muscle relaxants and barbiturates were targeted and quantified, with selected non-active metabolites qualitatively analyzed where appropriate. More so, limits of detections were further challenged and achieved to allow for the analysis of Drug-Facilitated Crime/Sexual Assault casework according to the even lower recommended LOD targets for such casework.

Discussion
In the City and County of San Francisco, all forensic toxicological examinations are performed law-enforcement-independently by the Office of the Chief Medical Examiner. This includes the testing of all samples from DUID casework who were observed to have signs of impairment for alcohol and drugs in all samples, regardless of the suspected impairing substance following roadside examinations. As such, this original dataset provides broad and reliable information of drug use in drivers within a Californian county over several years. Notably, cannabis detection rates, although high, were relatively consistent throughout the three years, before and after legalization of recreational use.

Recommended scope and limits of toxicological testing for DUID samples are seldom met by laboratories within California. Typically, only BAC testing takes place, or in some counties where the BAC is less than 0.15% or 0.08%, drug class-specific testing may be performed following immunoassay screening or suspected specific drug impairment. This potentially leads to drugs being undetected and does not provide a complete profile of impairing drugs in the driver, whilst also placing constraints on public safety initiatives for studies that attempt to determine the identification of drugs in drivers.

The analytical approach, described here, requires relatively low resources, provides laboratories a tool to manage the testing during increasing drug-impaired driving rates with varied drug use, and subsequently provides information for improved road safety through better policing strategies and policy making.

Conclusions
The observation of drug and alcohol trends over a three year period demonstrated high substance use on our roads when impairment is observed, and suggested a list of target drugs to be included in future testing schemes. A comprehensive and rapid LC-MS/MS method has been developed and fully validated to meet and often exceed recently updated recommendations.
Abstract ID  448
THC and CBD concentrations in serum following single and repeated administration of “light cannabis”.
Filippo Pirani
Filippo Pirani¹, Alfredo Fabrizio Lo Faro², Flaminia Pantano³, Roberta Pacifici⁴, Simona Pichini⁵, Francesco Paolo Busardò⁶
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²Unit of Forensic Toxicology, Section of Legal Medicine, Department of Excellence SBSP, Università Politecnica delle Marche, Ancona, Italy.,
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Aim
“Light cannabis” is a product legally sold in Europe with Δ 9-tetrahydrocannabinol (THC) concentration lower than 0.2% and variable cannabidiol (CBD) content. We studied THC and CBD concentrations in serum after smoking one or four light cannabis cigarettes. The aims of the study were to investigate:

- THC and CBD time courses in serum after smoking one 1.0 g light cannabis cigarette;
- THC and CBD time courses in serum after smoking four 1.0 g light cannabis cigarettes within 4 h (1 cigarette per h);
- Physiological and behavioral effects following single or repeated light cannabis smoking

Methods
Serum samples were obtained from six healthy “light cannabis” consumers after smoking 1g “Easy Joint® Seedless CBD 5.8” containing 0.16% THC and 5.8% CBD, as previously analytically verified in our laboratory, (Group 1) and from six other individuals after smoking four 1.0 g light cannabis cigarettes within 4 h (Group 2). Samples were collected in glass tubes before and 0.5, 1, 2, 3, 4 and 5 h after the last cigarette in both groups.

One mL sample was extracted with a liquid-liquid method; samples were evaporated to dryness, reconstituted and injected onto a gas chromatograph equipped with an autosampler and coupled to a mass selective detector, according to a previously published and fully validated method (Pacifici et al. Clin. Chem. Lab. Med. 2019).

Results
THC and CBD were detected in plasma from 30 min up to 4 hours after smoking one and four cigarettes. The highest THC and CBD concentrations were observed 0.5 h after one cigarette smoking in Group 1. Serum THC ranged from 2.7 to 5.9 ng/mL with a mean (SD) maximum value of 3.7 (1.2) ng/mL while serum CBD varied from 5.7 to 48.2 ng/mL with a mean (SD) maximum value of 23.7 (16.3) ng/mL. THC/CBD concentration ratio fluctuated between 0.21 to 0.44.

Similarly, the highest THC and CBD concentrations were observed 0.5 h after the smoking of the fourth cigarette in Group 2. In detail, THC range was 11.0-21.8 ng/mL, mean (SD) 23.5 (8.2) ng/mL and CBD was 19.2-35.3 ng/mL, mean (SD) 20.6 (4.0) ng/mL. THC/CBD concentration ratio fluctuated between 0.5 to 0.9.

Discussion
For the first time the kinetics of THC and CBD were investigated in serum following smoking of light cannabis cigarettes (<0.2% THC). THC and CBD showed a linear time course in serum with minimal THC concentration after a single cigarette smoking, while repeated smoking increased the values of one order of magnitude. CBD concentrations were higher than those of THC but did not linearly increase probably because CBD does not preferentially volatilize compared to THC. Limitations of the present study include the short total monitoring period of 5 h. However, all analytes were below the low LOQs (0.3 ng/ml serum) at 5 h, indicating that no quantitative data were missed. It has to be considered that light cannabis used in this study contains minimal amount THC and little amount GBD.

In addition, our first biological sample collection was at 0.5 h, after the Cmax that occurs just prior to the last puff on a cannabis cigarette.

THC and CBD time courses were quantified in serum after smoking four 1.0 g light cannabis cigarettes within 4 h (1 cigarette per h) measuring analytes concentrations in serum, in an authentic situation where multiple cannabis cigarettes are smoked within a short period of time. This is important to determine if multiple light cannabis cigarettes can produce positive cannabinoid tests, and to evaluate changes in physiological and subjective tests. There were no significant changes in blood pressure, heart rate and body temperature, following single and repeated light cannabis smoking up to 5 h post smoking. Group 2 participants went home at the end of the session and immediately fell asleep for at least 4 h as self-reported or reported by family or roommates.

Conclusions
In the study we reported, light cannabis was smoked in a controlled clinical trial; participants were cigarette smokers with a sporadic previous light cannabis experience. Serum THC/CBD concentration ratios never exceeded the value of 1.2 and it could represent a useful biomarker to distinguish the use of light cannabis vs. an illegal higher THC cannabis intake, where the THC/CBD ratios are generally greater than 10. Although these first observations did not provide evidence of serious side effects when smoking light cannabis, consumers should be advised of possible drowsiness after smoking.
Abstract ID 464
The emergence of deschloro-N-ethyl-ketamine, a ketamine analog, in drug seizures and drug driving cases in Hong Kong.
Wing Chi Cheng
Wing Chi Cheng, Kwok Leung Dao
Government Laboratory of Hong Kong

Aim
To describe the occurrence of deschloro-N-ethyl-ketamine (2-oxo-PCE), as a newly emerged drug in Hong Kong through examinations of drug seizures and blood samples from driving under the influence of drugs (DUID) cases.

Methods
As the only designated laboratory in Hong Kong responsible for forensic drug/toxicological examinations, all drug seizures (2705) and DUID (11) cases submitted from October 2017 to October 2018 were examined. Cases identified with 2-oxo-PCE, determined using validated method in compliance with ISO 17025 requirement, were included. Forensic drug testing in drugs seized were analyzed by dissolving the solid (~ 1mg) in ethanol using GC-MS/LC-DAD/GC-FID. 2-oxo-PCE and deschloronorkeetamine in blood were analysed using the method routinely used for analysis of specified drugs in DUID by LC-MS/MS through additional monitoring MRM transitions at m/z 218>173 and 218>91 for 2-oxo-PCE; and m/z 190>145 and 190>91 for deschloronorkeetamine. Blood samples (100 µL) was extracted with 1 mL of CH2Cl2/toluene/IBA mixture (3:6:1 v/v) after addition of Trizma buffer (pH 9) (200 µL) and a mixture of internal standards containing ketamine-d4. The organic extract was reconstituted into ammonium formate buffer (pH3) before LC-MS/MS analysis.

Results
The MDL, PQL, precision and recovery were 5 µg/mL, 12 µg/mL, 6.1% and 99% respectively for 2-oxo-PCE determination in drug seizures. With a calibration of 20 – 400 ng/mL, the LOD and LOQ for 2-oxo-PCE in blood were 0.22 ng/mL and 0.62 ng/mL respectively, the precision and accuracy at three different concentrations spiked in seven different blood matrices were <20% and <25% respectively.

Of the 2705 cases submitted in October 2017-October 2018, there were 31 cases (52 items) found with 2-oxo-PCE with physical appearance (mostly powdery or crystalline solid) similar to those ketamine seizures (352 cases in the same period). 2-oxo-PCE was mostly found as the sole (36 items, 69%) or major ingredient as a mixture with ketamine (10 items, 19%) in the samples. The purities of 5 samples with only 2-oxo-PCE found were in the range of 1.6-12.8% w/w. The 2-oxo-PCE (ketamine) contents in 2 samples found to mixed with ketamine were 18.6% (2.3%) and 30% (58.9%). The drug contents (1.6-30%) found in 2-oxo-PCE samples were significantly lower than the average ketamine content [mean: 81.1±22.6%] found in drug seizures.

The Police has been empowered to require a driver to undergo impairment test if the involvement of alcohol for the impairment is excluded by conducting a breath test. For those who fail the tests would be required to take blood/urine for toxicological examinations. Of a total of 11 DUID cases submitted in the study period, 4 cases were detected with 2-oxo-PCE. The results were summarized as follows:

<table>
<thead>
<tr>
<th>Case No.</th>
<th>2-oxo-PCE/deschloronorkeetamine (g/mL)</th>
<th>Other findings(g/mL)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.11/0.072</td>
<td>Unconscious; vehicle parked on roadside</td>
<td>Ketamine &amp; norketamine (&lt;0.01)</td>
</tr>
<tr>
<td>2</td>
<td>0.31/0.090</td>
<td>Unconscious; vehicle parked on roadside powder containing 2-oxo-PCE found in vehicle</td>
<td>Paracetamol (0.7)</td>
</tr>
<tr>
<td>3</td>
<td>0.08/0.038</td>
<td>Involved in traffic accident; emotionally unstable powder found in his nostrils</td>
<td>Benzoylecgonine (0.08)</td>
</tr>
<tr>
<td>4*</td>
<td>0.10/0.092</td>
<td>Involved in traffic accident powder found in nostrils</td>
<td>Paracetamol (1.2)</td>
</tr>
</tbody>
</table>

*Driver injured, no impairment assessment performed

Discussion
All 2-oxo-PCE seizures were mostly packaged in plastic bags or paper packets without labels. With considerable amount of 2-oxo-PCE (1.6-12.8% w/w) found in the samples though significantly lower than those found with ketamine (81.1±22.6%), it is unlikely for 2-oxo-PCE as an additive or impurities in the samples, and that its emergence as a substitute of ketamine is speculated. Given the seizures were physically similar with those of ketamine, the users might have been taking 2-oxo-PCE unknowingly as if they were taking ketamine.

In the 4 DUID cases, all drivers were male, ages 34-38, with both 2-oxo-PCE and deschloronorkeetamine, a metabolite via deethylation of 2-oxo-PCE, found in the blood. 3 out of 4 apprehended drivers presented with either impaired consciousness (cases 1 and 2) or emotionally unstable (case 3) at the time of incidents, consistent with reported 2-oxo-PCE intoxicated symptoms in local hospitals. For case 4, the driver was injured without preliminary assessment but was involved in erratic driving (i.e., traffic accident). The finding of 2-oxo-PCE powder (case 2) and the powder found in nostril of a driver (case3) indicated nasal insufflation would be a possible route of administration. The blood 2-oxo-PCE concentrations (0.08-0.31 mg/mL) were generally lower than the reported ketamine levels in DUID cases.

Conclusions
The emergence of 2-oxo-PCE, a ketamine analog, as a new street drug encountered in Hong Kong is reported. The drug, sold with physical appearance similar to street level ketamine but having much lower drug purity.

The blood concentrations of 2-oxo-PCE and its metabolite, deschloronorkeetamine, were reported for the first time in DUID cases, indicative of their use of 2-oxo-PCE. With the lower blood drug concentrations found in the “intoxicated” drivers in comparison with reported levels of ketamine, it is not unreasonable to infer that 2-oxo-PCE would be more potent than ketamine as lower dosages of 2-oxo-PCE consumed would result in more severe signs of impairment.
Abstract ID 492
Large discrepancies of methamphetamine immunoassay screening and GC-MS confirmation results caused by presence of optically pure R-(-)-MA in serum samples.
Lisa Oóowski
Lisa Oóowski, Grit Kießling, Julia Dinger, Frank Theodor Peters
University Hospital Jena, Department of Forensic Medicine

Aim
Introduction:
The psychostimulant drug methamphetamine (MA) is widely abused in the German federal states Saxony, Saxony-Anhalt, Thuringia and Bavaria (parts). As a chiral compound, methamphetamine exists in the form of two optical isomers, namely R-(-)-MA (L-methamphetamine) and S-(+)-MA (d-methamphetamine). The latter has more potent stimulant effects and a higher misuse potential, but a shorter plasma half-life than the R-(-)-enantiomer. In Germany, illicit MA sold as “crystal” or “ice” used to contain almost exclusively the hydrochloride of optically pure S-(+)-MA synthesized from ephedrine or pseudoephedrine with occasional occurrences of racemic MA.

Recently, a serum sample from a DUID case tested positive in the authors’ laboratory using the Methamphetamine Microplate EIA Kit (Diagnostik Nord, Schwerin Germany), the result being just above the cut-off value of 10 ng/mL. However, non-enantioselective confirmation by GC-MS after liquid-liquid extraction and heptafluorobutyrylation yielded a MA concentration of ca. 1100 ng/mL. As a potential explanation observation, it was hypothesized that the MA present in this particular sample could be the optically pure R-(-)-enantiomer, which has poor cross reactivity with the above-mentioned immunoassay.

Objective:
The aim of the presented work was firstly to identify which enantiomer(s) of MA was/were present in the above-mentioned sample to confirm or refute the above hypothesis and secondly to do the same in retrospectively identified samples with large discrepancies between EIA and GC-MS confirmation results.

Methods
Method:
A total of 1114 cases with confirmed positive results for MA from the beginning of 2018 to present were reviewed with respect to the following criteria: (1) MA concentration > 25 ng/mL as determined by routine non-enantioselective GC-MS. (2) percent difference between routine EIA results for MA and routine non-enantioselective GC-MS results of at least 90%. Serum samples fulfilling these criteria (including the one mentioned above) were included in the study.

All routine EIA results had been acquired with the Methamphetamine Microplate EIA Kit (Diagnostik Nord, Schwerin Germany) run on a DSX-Automated ELISA System (Dynex Technologies, Chantilly USA) and confirmed with a fully validated and accredited quantitative non-enantioselective method employing liquid-liquid extraction, derivatization with HFBA and GC-MS analysis in SIM mode. In addition to these routine analyses, all included serum samples were analyzed using the CEDIA Methamphetamine Assay OFT (Thermo Fisher Scientific, Dreieich Germany) run on an AU 480 instrument (Beckman/Coulter, Krefeld Germany). Finally, the samples were submitted to enantioselective GC-MS analysis after liquid-liquid extraction and chiral derivatization with S-trifluorobutryl prolyl chloride (S-TPC).

Results

Results:
In the 1114 retrospectively reviewed cases EIA results for MA were on average 26% higher than those of non-enantioselective GC-MS analysis (range -200% to +185%). From the respective serum samples, 16 fulfilled the defined acceptance criteria. For four of the samples no material for further analysis was available. Enantioselective reanalysis of the remaining 12 samples showed that seven of these samples contained optically pure S-(+)-MA, and all of them were tested positive by both immunoassays. Five samples contained optically pure R-(-)-MA. None of the samples contained racemic MA. Despite fairly high MA concentrations in the five R-(-)-MA samples (110 – 1100 ng/mL), only two of them were tested positive by both EIA and CEDIA. Two further samples were only slightly above the cut-off in EIA and one sample was tested negative by both immunoassays.

Discussion

Discussion:
The detection of optically pure R-(-)-MA in five routine serum samples indicates that R-(-)-MA has become available as a new form of MA on the drug market. Although it cannot be fully excluded that the detected R-(-)-MA could have been present as a metabolite of the antiparkinson drug selegiline, this seems very unlikely considering the high R-(-)-MA in four out of five samples. Moreover, a recent drug seizure by the Thuringian State Criminal Police Office was found to contain optically pure R-(-)-MA. This finding is of relevance, because R-(-)-MA – like S-(+)-MA and racemic MA – is listed as controlled substance in Germany under the name of levmetamfetamine. However, the results of the presented study indicate, that it is poorly picked up by the used immunoassays and most likely by other immunoassays as well because the antibodies of these tests generally target S-(+)-MA. The classical analytical strategy with immunoassay-based screening followed by confirmation using hyphenated techniques should therefore be associated with a considerable risk of false negative findings with respect to R-(-)-MA, especially if present at relatively low concentrations.

Conclusions

Conclusions:
The recent appearance of R-(-)-MA on the illicit drug market and consequently routine drug testing cases has consequences for the drug testing strategy. Firstly, it has to be ascertained that this enantiomer is covered by the screening technique in order to prevent false negatives. Secondly, confirmation enantioselective methods should be preferred for confirmation analysis.
Abstract ID 502
Application of Hair Analysis to Document Illegal 5-Methoxy-N,N-diisopropyltryptamine (5-MeO-DiPT) use.

Ping Xiang
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Academy of Forensic Science, China

Aim
5-Methoxy-N,N-diisopropyltryptamine (5-MeO-DiPT) is a designer hallucinogen that is a synthetic tryptamine derivative. It is highly abused and is involved in criminal activities because of its psychotropic properties. Herein, we presented an UHPLC-MS/MS method allowing for the quantitative determination of 5-MeO-DiPT in human hair.

Methods
The hair samples were rinsed three times with acetone before extraction. The sample was allowed to dry at room temperature. After drying, the hair was cut to a 1-2 mm pieces and stored in paper envelopes until analysis for method validation. Aliquots of 30 mg hair pieces were weighed and placed in a 2-mL tube containing ceramic beads; 0.5 mL IS Psilocin-D10(2 ng/mL, prepared by dilution in formic acid:water (v/v 1:1000)) and calibrant solution were added successively. Then, the samples were placed on the BeadRuptor system (OMNI, USA) to pulverize below 4°C, using the following procedure: speed, 6 m/s; time, 20 s; dwell, 40 s; repeated 10 times. The mixture was centrifuged twice and centrifuged at 14,100 g. Finally, 200 μL supernatant was pipetted into autosampler vials, and 5 μL was injected into the LC-MS/MS system. A T3 column (100 mm×2.1 mm, 1.8 μm) was used, and mobile phases consisted of 20 mmol/L ammonium acetate, 5% acetonitrile and 0.1% formic acid in water (solvent A) and acetonitrile (solvent B). The gradient elution was used at a flow rate of 0.3 mL/min. Method validation was applied in the present study according to the recommendations of Peters’, including selectivity, linearity, limits of detection (LOD), lower limit of quantification (LLOQ), accuracy, precision, matrix effect and recovery.

Results
The resulting calibration curve for 5-MeO-DiPT was y=281.50213x+0.00231 (R2=0.992), the limit of detection (LOQ) was 0.05 pg/mg, and the lower limit of quantification (LLOQ) was 0.1 pg/mg. The accuracy was between 92.1% and 105.6%, and the within- and between-day precision, recovery and matrix effect were acceptable. The validated method was successfully used in 106 real drug abused cases, and the concentration of 5-MeO-DiPT in hair samples of these suspected users was 0.2-7532.5 pg/mg. These cases present data to document illegal 5-MeO-DiPT use.

Discussion
Sex difference is the obvious characteristics among the 106 suspected 5-MeO-DiPT users; only two of 106 cases were female, and the concentration of 5-MeO-DiPT of these females was < LLOQ. The 30-40-year-old age group was the major population that used 5-MeO-DiPT because this group is in their prime, which means they have money and chances to come into contact with the new drugs. Besides, 5-MeO-DiPT and MDMA have similar disruptive effects to central serotonergic systems. Thus, it is important for us to pay attention to the drug combination in the study of forensic toxicology.

Conclusions
A sensitive and reliable quantitative method was established for the detection of 5-MeO-DiPT in hair samples. The validated method was successfully used in 106 real cases, and the concentration of 5-MeO-DiPT in hair samples of these suspect users was 0.2-7532.5 pg/mg. These cases present data to document illegal 5-MeO-DiPT use.
Abstract ID 512
Predominance of illicit drugs and ethanol in blood among suspected drug-impaired drivers in Western Switzerland.
Marc Augsburger
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Aim
In order to gain more information about the type of drugs used by drivers and their blood concentrations, we analyzed 1573 blood samples of drivers suspected of driving under the influence of drug (DUID) within a 12 months period in Western Switzerland.

Methods
All official DUID cases submitted by the Justice during one year (2018) concerning living individuals were considered. Post-mortem cases were not considered. This study included 1360 men (86%) and 213 women (14%) controlled by the police or involved in a crash. Police officers were allowed to perform drugtest on-site, such as saliva tests (Drugwipe), however results of these tests could not be considered for prosecution. The average age of the drivers was 34±14 years old (minimum 13 and maximum 95). Blood samples (4 tubes of 5 ml with NaF/EDTA, and 1 tube of 9 ml with EDTA) and urine samples were sampled by a medical doctor, who evaluated driving impairment when possible. For all cases, general unknown screening were performed. Drug screening in urine and/or blood samples was performed by immunoassays and by gas chromatography coupled to mass spectrometry (GC-MS) and/or liquid chromatography coupled to high resolution mass spectrometry (Orbitrap) (LC-HRMS), including illegal, such as cannabinoids, cocaine, amphetamines, opiates/opioids or new psychoactive substances (NPS), and medicinal drugs, such as benzodiazepines, antidepressants, neuroleptics, psychostimulants, antiepileptic drugs or sedatives. Ethanol quantification was performed by head-space gas chromatography coupled to flame ionization detection (HS-GC-FID) (LOQ: 0.10 g/kg). Other drugs were quantified by GC-MS or LC-MS/MS using validated methods with different LOQ for each substance.

Results
One or more psychoactive drugs were detected in 88% of blood samples. The detected drugs in blood were cannabinoids (57%), ethanol (29%), cocaine (21%), benzodiazepines (11%), opiates/opioids (8.5%), amphetamines (6.7%), antidepressants (5.0%), neuroleptics (1.0%), and LSD (0.1%). Among these 1573 cases, the following substances (N; 5-percentile - median - 95-percentile in (ng/ml) excepted for ethanol in (g/Kg)) were detected: THC-COOH (888; 7.0 - 33 - 120), Δ9-THC (792; 1.3 - 5.0 - 20), CBD (352; 1.0 - 1.7 - 8.9), ethanol (437; 0.32 - 1.22 - 2.35), cocaine (199; 12 - 49 - 301), benzoylcegonine (328; 30 - 406 - 2500), free morphine (86; 11 - 44 - 318), methadone (42; 32 - 130 - 475), tramadol (15; 20 – 160 - 1350), O-desmethyl-tramadol (12; 8.6 - 35 - 44), amphetamine (65; 14 - 67 - 540), methamphetamine (28; 14 - 170 - 778), MDMA (53; 29 - 190 - 778), LSD (2; 0.41 - 0.45 - 0.50), alprazolam (22; 3.7 - 38 - 207), bromazepam (9; 20 - 155 - 368), clonazepam (9; 11 - 45 - 94), diazepam (11; 12 - 88 - 530), desalkyl-flurazepam (9; 15 - 54 - 362), lorazepam (33; 6.2 - 27 - 85), midazolam (58; 7.2 - 30 - 324), nordiazépam (31; 15 - 150 - 1130), oxazépam (38; 20 - 84 - 1126), zolpidem (24; 20 - 200 - 954), citalopram (31; 20 - 43 - 117), clomipramine (5; 31 - 83 - 160), fluoxetine (5; 24 - 28 - 264), mirtazapine (5; 24 - 52 - 71), sertraline (6; 68 - 110 - 214), trazodone (16; 82 - 400 - 958), venlafaxine (11; 45 - 97 - 610), quetiapine (14; 11 - 50 - 296), lamotrigine (5; 271 - 2700 - 6030), ritalinic acid (5; 21 - 94 - 225), and diphenhydramine (8; 47 - 95 - 151).

Discussion
In 1067 cases (68%), the concentration of at least one substance defined in Swiss Traffic Law presents a concentration equal to or higher than the legal limit (ethanol blood concentration: 0.50 g/kg; THC blood concentration: 2.2 ng/ml; free morphine, cocaine, amphetamine, methamphetamine, MDMA, MDEA blood concentration: 22 ng/ml). In comparison to a similar study conducted in 2002 and 2003 [Augsburger et al., Forensic Sci Int 153 (2005) 11-15], an increase of the prevalence of cocaine consumption was observed, as well as an increase of the median concentration of THC in blood, from 3 to 5.0 ng/ml, and the emergence of the consumption of hemp with high level of CBD. No substances classified as NPS were detected, suggesting a low prevalence of consumption of these substances in Western Switzerland. It must be emphasized that for other substances than ethanol, THC, free morphine, cocaine, MDMA, MDEA, amphetamine and methamphetamine, no cutoff are defined in the Swiss Traffic Law, and impairment must be evaluated by forensic experts taking into account the police report, the medical examination report and the report of toxicological blood analysis.

Conclusions
The majority of substances detected in blood of drivers suspected of DUID in Western Switzerland are illicit drugs and ethanol, as already observed in previous studies. For the majority of cases, the concentrations of drugs found in blood confirmed drugged-impaired drivers according to the Swiss Traffic Law.
Abstract ID 542  
The new trends of drug diversion and drug use in the Western Region of Algeria.  

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Aim  
Given its dynamic nature, the drug problem is a global challenge. Over the years, misuse and diversion of prescribed drugs has been a concern in Algeria. Drug diversion is a medical and legal concept involving the transfer of any legally prescribed controlled substances from a licit to an illicit use. This includes transferring drugs to people they were not prescribed for. The aim of this study is to evaluate recent trends in the use and misuse of drugs.

Methods  
Drug use profile: It is a retrospective study of the profile of drug use in the last five years in the department of pharmacology and toxicology of Oran University Hospital. Toxicological analysis were performed by enzymatic method and immunochromatographic rapid tests for screening of drugs in urine. multiple samples were taken over regular time periods and tested for cannabis, benzodiazepines, amphetamines, cocaine and other drugs.

Drug diversion profile: The recent trends of drug diversion were evaluated through a preliminary cross-sectional survey of health professionals. The data was collected through questionnaires. The questions answered are to estimate who, what, how, how much, and for what purpose drug diversion occurs. In total, 218 healthcare professional (109 Pharmacist and 109 Physicians) have participated in this study.

Results  
The study of the profile of the screening tests of drugs showed that cannabis remains the most widely abused illicit drug. However, according to the statements of drug users, the change of the trends of drug use and drug diversion is noticeable. Indeed, the findings of the survey showed that along with great access to drugs commonly prescribed inevitably comes diversion outside a prescribed treatment plan. The phenomenon is increasing and its profile is changing over the years. According to the statements of health professionals, we note that currently Tramadol is the most diverted from its medical use (18%) followed by Pregabalin (17.5%) and Benzodiazepines (12%). Whereas, in few years ago Benzodiazepines had been the most diverted with Trihexyphenidyl; as a matter of fact, we have observed a significant decrease of the cases of positive screening test of benzodiazepines over the past five years.

Discussion  
The drug diversion phenomenon has recently been a problem. Often it is the pharmacist who first detects a diversion attempt. However, he doesn’t make systematically notifications or declarations of drug diversion, given the absence of an addictovigilance system in Algeria. Indeed, the tools of prevention and detection of drug diversion, and dealing with abusers, are insufficient. In the other hand, Physicians have to participate to identify current or previous addictive behaviours in all patients to whom potentially addictive drugs are prescribed.

Conclusions  
The findings of this study highlight the potential for misuse and diversion of drugs which involve awareness and management by installing an addictovigilance system and providing an expanding array of tools for ensuring medication adherence and reducing risk of diversion. Preventing drug diversion will require a long-term public health initiative. It is considered necessary to develop techniques to broaden the screening of drugs according to the currently drug use profile.
Abstract ID 68
Cognitive bias in forensic toxicology.

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Aim
Cognitive bias has been studied in many forensic disciplines, such as fingerprints, bullet comparison, and document examination. However, knowledge of cognitive bias within forensic toxicology is lacking. With this study, we aimed to explore how and when forensic toxicology casework is vulnerable to cognitive bias, raise awareness of it amongst forensic toxicologists, and prompt discussion. We also aimed to gather a list of potential bias-minimizing strategies from TIAFT members for best cognitive practice in forensic toxicology casework.

Methods
The authors (a forensic toxicologist and a psychologist) examined the different stages of forensic toxicology casework to determine what information is relevant to the task at each stage. We also surveyed TIAFT members at the 2016 conference in Brisbane, Australia about their experiences using contextual information, and asked for suggestions of potential bias-minimizing strategies. The study received ethical approval from the University of Glasgow (project number 200150170).

Results
We found that forensic toxicology casework involves subjective decision making, comparison, and interpretation; processes that are known to be vulnerable to cognitive bias. As with other fields, the impact of potential biases depends on the complexity of the case.

There are three points in a forensic toxicology case where bias can potentially be a problem:

1. Case strategy: At the start of the case, contextual information can be used transparently to determine which tests are appropriate (task-relevant). However, depending on the processes used, this stage may be vulnerable to expected-frequency bias. This is where experienced forensic toxicologists become accustomed to drug use occurring at certain rates among certain populations (e.g., by age, sexuality, cultural group, etc.), and therefore expect it to keep occurring at those rates. This may manifest itself in ‘rules of thumb’ being applied to cases, where for example, people over or under a certain age are treated differently.

2. Screening: In the lab, when a comparison is made between the case and a drug standard or MS library, contextual bias can occur if the case circumstances (e.g., diagnoses, prescriptions, evidence of drug use at the scene, etc.), are known by the analyst. In this situation, poor-quality chromatographic or mass spectral matches for drugs can be subconsciously ‘upgraded’ or ‘downgraded’ to fit the circumstances of the case.

3. Interpretation: During interpretation, case information is often task-relevant, however it can also sway or incline a forensic toxicologist towards only one interpretation of the test results, when in fact there are several possible explanations. Use of case circumstances during interpretation can also result in circumstantial evidence being double-counted, or the toxicology results being given more weight than they should by the trier of fact (judge, jury, Coroner, Medical Examiner, etc.)

There were no previously published bias-minimizing strategies for forensic toxicology casework, but 14 different suggestions came from TIAFT members based in 23 countries.

Discussion
No single bias-minimizing strategy has emerged from the cognitive forensics literature as being applicable in all situations or to all fields. As practices between forensic toxicology laboratories vary widely, we do not advocate a one-size-fits-all approach to best cognitive practice.

We did not record any potential bias-minimizing strategies for the case strategy step, however we note a move in many laboratories towards comprehensive screening methods (such as by LC-MS or LC-TOF-MS) for numerous drugs in one analysis, will reduce expected frequency bias.

Once the sample has entered the laboratory, TIAFT members suggested anonymizing the samples and/or restricting the availability of background information to analysts. Having strict scientific criteria for identification of a drug should also minimize the impact of contextual bias in the lab.

During interpretation, we do not advocate concealing contextual information, as this is often directly relevant to the task at hand. However, problems with bias can occur when circumstantial and toxicological evidence is combined in a way that is opaque. Models have been published recently that allow these two types of evidence to be combined in a transparent way (e.g., Nathan C. Stam et al., The attribution of a death to heroin: A model to help improve the consistent and transparent classification and reporting of heroin-related deaths, Forensic Science International, 2018, 281, 18–28). Forensic toxicologists should also be mindful that the information they receive on a case may be incorrect, missing or incomplete, out-of-date, or legally inadmissible.

Conclusions
This was the first study to examine cognitive bias in forensic toxicology, and it found that forensic toxicology casework is vulnerable to bias at three key stages: case strategy; screening; and interpretation. Bias-minimizing strategies have been suggested by TIAFT members, but need to be tested for efficacy and feasibility. We hope this study will provide an opportunity for us to critically examine our practices and workflow, and make improvements where applicable.

Reference
Abstract ID  82
Detection of Drug-Facilitated Sexual Assault (DFSA) compounds from adulterated beverages using monolithic silica adsorbents and multimode inlet GC-MS/MS.

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Aim
Drug-Facilitated Sexual Assault (DFSA) continues to be a social problem in Japan and all over the world. DFSA incidents often occur with the perpetrator spiking a beverage with a drug or other compound and offering the beverage to the unknowing victim. The ability to quickly analyze an adulterated beverage to determine its contents would be a powerful investigative tool. In Japan, there have been recent incidents of women being drugged with commonly available pharmaceutical medications, including anti-anxiety and sleep medications. In this study, we investigated the analysis of beverages like coffee, green tea, and several different alcoholic beverages adulterated with common anti-anxiety and sleep medications. The medications were selected from some actual cases that we have experienced, as well as related compounds. Monolithic material sorption extraction (MMSE) was utilized to extract and concentrate the compounds directly from the spiked liquids. The adsorbent containing the compounds was then placed in a glass liner and inserted into a multimode inlet system specialized for thermal desorption. Using these techniques, it was possible to quickly analyze the contents of spiked beverages without a time-consuming extraction and the use of organic solvents.

Methods
Reference standards for brotizolam, etizolam, flunitrazepam, zolpidem, and zopiclone were purchased from Sigma-Aldrich, and the monolithic adsorbent, MonoTrap® TD rods, and the reference standards for brotizolam, etizolam, flunitrazepam, and zopiclone were purchased from Sigma-Aldrich, Reference standards for brotizolam, etizolam, flunitrazepam, and zopiclone were purchased from Sigma-Aldrich, Shimadzu Corporation (Kyoto, Japan). Two types of monolithic adsorbent rods were investigated, the silica gel-ODS (RSC18TD) and graphite carbon-PDMS (RGPSTD) types. The beverages used for this study were either purchased (alcoholic beverages) or were available in our department (coffee, green tea). All other chemicals and compounds were reagent grade. A 0.2 mL aliquot of beverage was added to a 2-mL plastic tube, followed by 5 μL of a 100 μg/mL working standard solution containing the 5 reference compounds. A 10 μL aliquot of an internal standard solution was also added. After the addition of 1.8 mL of distilled water, a monolithic adsorption rod was added, the tube sealed and vortexed for 10 min on a mixer. After vortexing, the rod was removed and patted dry with a clean wipe, inserted into a specialized glass liner, and desorbed in a multimode inlet for GC-MS/MS analysis. The multimode inlet used for thermal desorption, the Optic-4 multimode GC inlet, was kindly lent to our department by Shimadzu Corporation (Kyoto, Japan). Gas chromatography-tandem mass spectrometry (GC-MS/MS) analysis was achieved on a Shimadzu GC-MS-TQ8030. A tandem column comprised of a ZB-SemiVolatiles column (2 m x 0.18 mm i.d. x 0.5 μm; Phenomenex) coupled to a BPX5 column (4m x 0.15mm i.d. x 0.25 μm; SGE) connected by a SilTite®-Union connector (SGE) was used for separation. The oven temperature program was initially 100°C for 1.5 min, ramped to 320°C at 50°C/min, and held for 2 min. The total program time was 7.9 min. The mass spectrometer was operated in the electron impact (EI) mode at 70 eV, with acquisition in both the scan mode (m/z 45-550) and the Multiple Reaction Monitoring (MRM) mode for selected compounds. Injection was made using the Optic-4 multimode inlet, with an initial injector temperature of 100°C for 30 s, 10°C/s to 300°C, and held for 60 s. Helium was the carrier gas at a constant flow of 4 ml/min and a split flow of 20 ml/min.

Results
Peaks for the 5 standard compounds and 8 internal standard compounds were confirmed in most of the beverages tested. Retention times for the IS and standard compounds ranged from 2.8 min to 5.4 min. Recovery varied between the silica gel and graphite carbon rod types, but most compounds were retained better using the graphite carbon type. Flunitrazepam was clearly detected from a 125 mL coffee sample in which a 1 mg tablet of Rohypnol was dissolved. An actual case sample of a non-alcoholic beer spiked with brotizolam was also analyzed and confirmed.

Discussion
Thermal desorption using a multimode inlet is a novel tool that has been used to analyze a wide variety of sample forms, but this is the first attempt to apply it to the practical analysis of forensic samples. Simplified pretreatment using the monolithic adsorbents was applied to lessen the effect of interfering compounds like sugars from the beverages. These sorbents were able to trap the compounds of interest within the PDMS phase coating the monolithic skeleton or by the graphite carbon present in and around the silica. Direct heating inside the thermal desorption inlet allowed for simple and rapid analysis of the rods after extraction. Detection by tandem MS maximized sensitivity and minimized the effect of interfering substances. These techniques could potentially be applied to direct analysis of human samples.

Conclusions
A novel and rapid analysis method was developed using monolithic material sorption extraction and thermal desorption with a multimode GC inlet. This new method could prove useful in the detection of drugs from beverages used in DFSA.
Abstract ID  92
Fast UHPLC-MS/MS screening for 87 NPS and 32 classic illicit drugs in whole blood, serum, urine and hair.
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Aim
New Psychoactive Substances (NPS) detection is a current major challenge in international scenario of clinical and forensic toxicology. The permanence on the street and web markets and their commercial price mainly depends on “consumers satisfaction” in term of the balance between number and length of positive subjective effects vs eventual side effects. Whereas these two latter items can be reported in web, acute intoxications requiring hospitalization and fatalities are not immediately available to consumers and often NPS users are not aware of the most threatening consequences of NPS.

In this concern, the prompt analytical identification of newly introduced NPS is affected by the unavailability of rapid screening tests to detect them in emergency departments and by the paucity of feasible confirmatory analytical procedures and of certified reference materials. We aimed to develop and validate a fast and simple ultra-high-performance liquid chromatography-tandem mass spectrometry screening to identify and quantify 87 among the most abused NPS and 32 classic illicit drugs in whole blood, serum, urine and hair.

Methods
Blood samples were pretreated according to the following procedures: to 100 µl of whole blood spiked with internal standard deuterated mix, 70 µL M3’ and 500 µL acetone: acetonitrile 8:2 (v/v) were added. After vortexing and centrifuging, supernatant was evaporated to dryness under nitrogen at 45°C. Samples were reconstituted with 1 mL mobile phase, centrifuged and 1 µL supernatant was injected into the chromatographic system. Serum and urine samples were pretreated according to the following procedures: to 100 µl of sample spiked with internal standard deuterated mix, were added 500 µl of M3’ reagent (Comedical’) and 1µL injected into the chromatographic system. In case of hair, after washing twice with dichloromethane, 25 mg sample was finely cut and incubated with 500 µl of M3’ reagent (Comedical’) for 60 minutes at 100°C. After cooling, 1 µL solution was injected into the chromatographic system. LC analysis was carried out with an UPLC Waters Acquity I Class instrument coupled with a Waters XEVO TQ-S Micro tandem mass spectrometer. The reversed phase column used for analytes separation was an Oasis HLB (5 µm 4.6 x 20 mm), set at the temperature of 50°C. A run time of 10 minutes with a gradient mobile phase composed by ammonium formate 12.5 mM pH 9.5 (mobile phase A) and acetonitrile (mobile phase B) at the flow rate 1ml/min was selected. Initial conditions were 75:25 (A:B). Phase A was gradually ramped down from 75 to 0% and phase B gradually ramped up from 25 to 100%.

Mass spectrometric analysis was performed in positive ion MRM mode. Blank whole blood, serum and urine were used to prepare standard calibration curves, obtained with fortification with certified standards and certified deuterated standards for each analyte.

The method was fully validated according to the Guidelines provided by Peters et al. (Forensic Sci Int. 2007). The calibration range for hair was from 50 to 1000 pg/mg, that for blood and serum was 5 - 1000 ng/mL while that for urine was 50 - 1000 ng/mL. The method was linear for all analytes under investigation with a r2 always better than 0.99. Limits of quantification ranged from 10 to 30 pg analyte per mg hair and from 0.2 to 6 ng analyte per mL whole blood, serum and urine. Recovery of analytes under investigation and matrix effect were always higher than 80% whereas intra-assay and inter-assay precision and accuracy were always better than 15%.

Results
Application to real samples.
Hair samples
The developed method was successfully applied to hair samples from 8 cases tested positive for NPS and classic illicit drugs. All samples were positive for one or more targeted analytes. 7 cases were positive for cocaine and methadone. 3 cases were positive for fentanyl analogues and 5 cases were positive for 5-methoxy-α-methyltryptamine (5-Meo-aMT), the synthetic cannabinoid UR-144, 6-(2-aminopropyl) benzofuran (6 APM), APP FUBINACA and mephedrone, respectively.

Blood, Serum and Urine samples
42 postmortem blood, urine, or hair specimens from 27 fatalities were available. 17 blood and serum samples were positive for fentanyl analogues and metabolites and 3 samples were positive for classic illicit drugs such as morphine, codeine and oxycodone. 25 urine samples were positive for fentanyl analogues and metabolites and one sample was positive for methylphenidate and ritalinic acid. Finally, 14 samples were positive for classic illicit drugs such as nordiazepam, noroxycodone, 6-MAM, morphine and codeine.

Discussion
The presented UHPLC-MS/MS method allows the screening of several NPS and “classic” drugs of abuse on biological samples on intoxicated individuals and decedents. The developed method allows a rapid analysis time and requires a minimum amount of biological specimen.

Conclusions
The current method can be easily expanded to include a greater number of new psychoactive substances and can be used in high throughput clinical and forensic laboratories where a high number of samples have to be analyzed saving time and economic resources.
Abstract ID 97
Research on screening methods of common hypnotic drugs and metabolites: application to forensic cases.
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Aim
More and more hypnotics are used to treat insomnia. Since hypnotics are often used by criminals, efficient analytical methods are needed for detection in forensic cases. In recent years, the metabolite analysis in these hypnotics cases has become more and more important. A screening liquid chromatography tandem mass spectrometry method in combination with a simple and efficient sample preparation was established for the determination of 30 kinds of target substances including non-benzodiazepine hypnotics, benzodiazepine hypnotics, melatonin and other high-frequency drugs and their metabolites. Such as zopiclone, zaleplon, zolpidem, clonazepam, lorazepam etc. The method had been used and played a bigger role in forensic cases. Lots of toxicological data of hypnotics poisoning were collected through many cases studies.

Methods
Biological liquid samples was extracted by 0.1% acetonitrile formate and dehydrated with anhydrous sodium sulfate. This sample preparation method was simple, fast, effective. After preparation, blood and urine samples were detected under a multiple reaction monitoring (MRM) mode by ultra performance liquid chromatography tandem electrospray ionization triple quadrupole-mass spectrometry (UPLC-TQ/MS). Numerous parameters such as ionization, mobile phase, LC column can have a major impact on method performance. These parameters were optimized to improve the sensitivity and selectivity of a LC-MS/MS method. These targets hypnotics were separated on Waters BEH C18 column by gradient elution with 0.1% formic acid-0.1% formic acid/acetonitrile as mobile phase, ionized with positive eletrospray (ESI+). For each hypnotics, the two daughter ions with the strongest signals were paired with respective parent ions, as the indicator of qualitative analysis, and the pair with the strongest signal was used as the indicator of quantitative analysis.

Results
The method was validated to show high accuracy, linearity, and correlation coefficient (R2>0.99). The limit of quantitation (LOQ) varied from 0.1 to 1 ng/mL (S/N=10). The limit of detection (LOD) were 0.01-0.05 ng/mL (S/N=3). The average recovery rate was higher than 72%, and the RSD of intraday and interday were less than 15%. No interferences were found in blank samples. This proves the high selectivity of the method. Identification criteria was retention time provided by the combination of two transitions and their relative abundances. Each analysis time takes less than 5 min. This method was successfully used to detect and solve many difficult cases. Many hypnotic drugs and their metabolites had been found in different cases, such as zaleplon and 5-oxy-zaleplon, zolpidem and zolpidem carboxylate, clonazepam and 7-aminoclonazepam, etc. These information provided effective clues to the cases.

Discussion
A part of hypnotics pharmacodynamics absorbed fast, had a quick onset and a short half-life. For example, zolpidem usually takes effect in 15 minutes and has a 2~3-hour half-life. These hypnotics with its short half-life are not easy to detect in forensic cases also due to delay in going to the police, low concentration, and short window of detection. But the metabolite can offer particular advantages because a larger window of detection. The accuracy of the method has been verified in the detection of many cases, by investigating linearity, precision, detection limit, recovery rate and matrix effect. On the one hand, the method can solve the problem of low concentration and easy metabolism of the parental drugs. On the other hand, in combination with the information of metabolites and parental drugs in biological samples can improve the accuracy of test results. In two cases of anaesthesia rape, clonazepam and its metabolites 7-aminoclonazepam were detected in the urine of one female victim after 2 days, and the low concentration of 7-aminoclonazepam metabolites was also detected in the urine of the 2 victims after 5 days. In an anaesthesia robbery case, zaleplon and 5-oxygen zaleplon metabolites were detected, but desethylzaleplon was not found. This result was different from medical reports. In a blind screening case, midazolam and hydroxymidazolam, zolpidem and two carboxylic acid metabolites were detected in the body of the deceased. In these cases, we can confirm which kind of hypnotics. Qualitative and quantitative tests of hypnotics and metabolites were carried out and the case was analyzed by the ratio of the concentration of hypnotics and metabolites. These data were collected and could be helpful for the interpretation in cases of intoxication. At the same time, these data can provide reference for the relevant toxicology information of human poisoning. The similar structure and chemical properties of the same class hypnotics may produce the same metabolites. Therefore, it is important to notice that there is no one-to-one correspondence when inferring the original hypnotics. Several benzodiazepine hypnotics may produce a metabolite call diazepam.

Conclusions
The method was simple, rapid and highly sensitive. It is suitable for routine analysis of forensic cases. And it can improve the positive detection rate of cases and the admissibility of evidence. It's very important to analyze detection results. In particular, the body metabolism is complex. It should be cautious that one metabolite trace back the original hypnotics, which should be determined by being determined as many metabolites as possible.
Abstract ID 151
Prevalence of drugs and alcohol in sexual assault cases and the interest of the toxicological analysis in the judicial investigation and final judgement.
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Methods

Aims:

Background: In 2018, 1538 charges of rape and 1712 sexual assaults were claimed in Belgium. If a forensic investigation is started, a medical investigation takes place and samples for DNA analysis, as well as blood and urine samples for a toxicological screening (STA) are taken. The National Institute of Criminalistics and Criminology receives about 300 Sexual Aggression Sets (SAS) each year. In 20% of the cases DNA analysis is required by the judicial authorities, while only 10% of the cases undergo a STA. The UNODC guidelines for adequate analysis of Drug Facilitated Crimes state that it is of importance to evaluate the drug intake, either voluntarily or administered on the behavioral capacities of the victim. The impact of DNA analysis on casework is known by prosecutors, however, the impact of a toxicological analysis on the judgment remains unknown. In cases of word of the suspect against word of the victim concerning mutual consent, or sexual assaults between (ex)-partners often the judicial authorities will dismiss the claim directly. In these cases, however, STA could result in more information for their final judgment.

Aims: to determine the prevalence of alcohol and drugs in sexual assault cases (DFSA) (1), to create an awareness and elaborate the possibilities in DFSA related forensic research (2), to evaluate the potential impact of the toxicology results on the judicial outcome (3).

Methods: DFSA in which the sampling of a SAS-kit was requested by the judicial authorities in Antwerp during 2017-2018 (n=117) were selected. These samples were analysed for ethanol via a GC-FID method, gammahydroxybutyric acid (GHB) via GC-MS after alkylation/acytlation with TFMA/HFB-OH and screened with high resolution mass spectrometry (LC-HRMS) via the Xevo-G2-QTOF-XS (Waters, Manchester, UK) with UNIFI software. Compounds detected via the LC-HRMS method were quantified via UPLC-tandem mass spectrometric techniques (MRM mode).

Results

Results and discussion: At the moment 38 DFSA are already investigated. SAS were taken 1 to 68 hours after the alleged facts. This delay is of utmost importance, as the obtained toxicological results can be biased because of drug and/or alcohol elimination. In addition, long delays can complicate interpretation due to intake of substances after the facts and before sampling. In 39% of the cases, drugs and/or alcohol were present in significant amounts in blood, potentially resulting in an altered awareness and thus incapacitation. In the blood samples 39% had poly-drug use (medication and/or drugs) or drug use combined with alcohol, while in 43% of the urine samples poly-abuse was detected. Twenty-four percent of blood samples contained ethanol (median 1,09 g/L, 0,11-2,51 g/L), 8% contained cocaine (median: cocaine 0, benzoylcegonine (BZE) 234, methylecgonine (ME) 10, cocaethyleen (CE) 3,9 ng/mL, range: 0-25 ng/mL cocaine, 155-354 ng/mL BZE, 8,5-28 ng/mL ME, 3,4-47 ng/mL CE ), 1 sample contained methylenedioxymethamphetamine (MDMA) with a concentration of 424 ng/mL and 2 samples contained 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH) with concentrations of 5.1 and 8.9 ng/mL. The blood samples also contained 11% benzodiazepines (zolpidem, alprazolam, diazepam, nordiazepam), 11% neuroleptic compounds (quetiapine, aripiprazole, amisulpride), 18% antidepressants (sertraline, trazodone, citalopram, fluoxetine, dosulepine), 5% tramadol and 37% contained other types of medication (eg. methylphenidate). Background information for the toxicologist is scarce and in only one case the victim admitted to have smoked a joint, but only 4 days before the alleged assault. In three cases medication use was admitted and in four cases alcohol use. Looking into our STA results, it is clear that victims often not reveal their (in)voluntary drug or alcohol intake. This information is taken into consideration by the judge, certainly in mutual-consent discussions. In one particular judgment the finding of (back calculated) 3 g/L blood-ethanol lead to a modification of the trial outcome as the judge decided that this would result in an incapacity of the victim to give consent. In a case in which the victim claimed to be drugged, the judge requested a hair analysis of the victim after the STA demonstrated a high MDMA blood-concentration to make sure it was a single intake, and it was not. In another case with a diazepam-positive blood sample, the suspect was accused of drugging the victim, however, after advice from the toxicologist to look into the medical file, it became clear that this was administrated as a medical treatment shortly before the SAS-sampling.

Discussion

Conclusions: This small scale epidemiological study shows which compounds are most prevalent in DFSA in Belgium. In addition, via case examples, the interest of STA to prove incapacitation of the victim in judicial procedures with mutual-consent discussions is proven. However, more attention has to be paid to ensure a short sampling delay and to get insight into the medical treatment. When obtaining more accurate (medical) information and a better sampling protocol, the interpretation of the toxicological analysis and thus its applicability will even improve.
Abstract ID 189


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Aim
The ISO/IEC 17025, “General Competency Requirements for Testing and Calibration Laboratories”, is the management standard system applied to the laboratories for accreditation purposes, that is, a worldwide external recognition of “technical competence”. The standard has been revised and the new version was published in November 2017, with the transition process in the accredited laboratories being underway.

In the initial revision phase, the ISO/CASCO working group, and according to the guidelines established by QS-CAS-PROC/33 (August 2015), identified the requirements to be assessed, as the use of specific terminology in the requirement’s standard text related to impartiality, confidentiality, complaints and management system, in addition to the structural alignment with other references. In the 2017 edition, there are substantial differences in structure, namely in the concept of impartiality and confidentiality and in the identification of risks and opportunities. It also maintains its philosophy in terms of establishing general requirements focused on the competence, being, therefore, a gold standard for the laboratory community. It also clearly states that laboratories that are part of complex organizations may operate a management system complying with ISO/IEC 17025 and ISO/IEC 9001 standards, in an integrated approach.

Methods
The International Laboratory Accreditation Cooperation (ILAC) has established a three-year period for harmonization and implementation of actions required to meet the new standard’s requirements. This transition process is a major challenge for the laboratories, namely for the Forensic Chemistry and Toxicology Service of the Portuguese National Institute of Legal Medicine and Forensic Sciences, including the implementation of a transition policy and consequent establishment of a transition plan that comprises predictable adjustments in terms of material requirements and human and financial resources, accomplishing the defined deadlines.

Results
In this work, the authors present the Portuguese Forensic Chemistry and Toxicology Service perspective regarding the essential steps and chronology in order to allow the laboratory to have its management system aligned with ISO/IEC 17025:2017. This process, initiated in 2018, includes relevant documentation of the process, human resources requalification, a transition calendar suitable with the laboratory resources and funding, and an external audit as to the implementation process is concerned, thus maintaining its accreditation.

Discussion
The transition for the revised version of ISO/IEC 17025 is mandatory and necessary for the maintenance of accreditation by laboratories. During the expected transition period, it is important to note that both ISO/IEC 17025:2005 and ISO/IEC 17025:2017 are equally valid and applicable. This implies that, through this time, tight planning and control of the management system will be required to maintain focus and minimize the additional entropy naturally created in these transition processes.

Conclusions
With the transition to this new revision of the ISO/IEC 17025 standard, it is expected that the global laboratory community will be able to gain added value from this review process by strengthening its technical competence, maintaining the aim of rigorous and valid results, contributing to an increase in the society’s quality of life and safety, as a form of differentiation and, finally, guaranteeing its competitiveness and sustainability.
Abstract ID 238
The Unconventional side of Forensic Toxicology in the UK.
Mark Tyler
Mark Tyler
Eurofins Forensic Services

Aim
The aim of this presentation is to highlight the unconventional side of toxicology. A number of cases will be discussed involving non-routine exhibits. The presentation will include the associated challenges, analytical strategy and results of some “bespoke” exhibits, which formed part of the investigation into incidents such as spiked drinks, assault, adulterated food and drug facilitated sexual assault (DFSA) amongst others.

Methods
The modern Forensic Toxicology Laboratory is constantly challenged to evolve. This has brought about many improvements in methodology for analysing blood, urine, hair and other specimens, and also for the validation and accreditation of methods. Non-routine exhibits are challenging for various reasons, but by using standard forensic principles, including necessary blanks, negative and positive controls, and appropriate dilutions where relevant, toxicological examination of unconventional items can yield important results for the investigator.

Results
Case 1: Drug Facilitated Sexual Assault.
A rape victim had little memory of the incident and reported it several days afterwards. No blood, urine or hair samples were collected. The suspect was arrested many weeks later, and was in possession of some gel sachets (sildenafil and dapoxetine), tablets (possibly tadalafil) and a bottle of clear liquid. The liquid was analysed and found to contain GBL. The suspect claimed that sex occurred with consent, but was subsequently convicted of drug facilitated rape.

Case 2: Administration of a Noxious Substance.
A female stayed with her partner overnight, but awoke the following day feeling “groggy and unwell”. She found her bottle of lemonade which still contained some of the liquid lemonade, but with unexpected sediment. It was several days before the police were informed and the bottle was seized. No biological specimens were taken. Analysis of the liquid detected mirtazapine and buspirone (equivalent to absolute amounts of approximately 8 milligrams and 1.5 milligrams respectively in the remaining liquid).

Case 3: Food adulteration
The suspect made a cheese salad for his ex-partner. The victim ate some but it didn’t taste right and soon afterwards she felt tired. She believes the suspect added her mother’s sleeping tablets (“Dormicum”, or midazolam, 15 mg) to the salad. Analysis of the remaining salad detected midazolam at an absolute amount equivalent to approximately 1/3 of one 15 mg tablet. The suspect admitted the offence.

Case 4: Assault.
Police Officers were called to the scene of an assault between 2 males. The victim had a serious head injury and was treated at the scene. Large head bandages were applied which became heavily blood stained. The suspect stated that the victim had been abusing methylamphetamine. The victim refused to give blood or urine. The bandages were seized. An unstained area of the bandage was analysed for drugs and was negative. A blood-stained area was analysed and tested positive for methylamphetamine and amphetamine.

Discussion
It is impossible to validate and accredit methods for all exhibits and eventualities. Such items are often time consuming and may require a large amount of space, and are a challenge if resources are limited. However, the examination of the unconventional exhibit provides added interest in the laboratory, and can be important for training and developing the next generation of toxicologists, allowing them to understand the potential opportunities and associated limitations, and encouraging good laboratory practice.

Conclusions
Unconventional toxicology items will often be submitted to the laboratory and will present their own unique challenges, but there is much to be gained from their examination, providing valuable results to the investigators and enhancing the knowledge and experience of the toxicologist.
Abstract ID 359
Pharmacological evaluation and detection of the recently emerged synthetic cannabinoid 4F-MDMB-BINACA in ‘legal-high’ products and human urine specimens.

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Aim
Synthetic cannabinoids remain one of the largest groups of novel psychoactive substances (NPS) in the European drug market. Frequently emerging NPS are challenging for laboratories performing forensic drug analysis in biological matrices. The newly emerged synthetic cannabinoid (SC) 4F-MDMB-BINACA (syn. 4F-MDMB-BUTINACA) has been reported by different law enforcement agencies all over Europe and the USA since November 2018. Distribution of 4F-MDMB-BINACA on the German drug market and frequent consumption were observed since August 2018. This work aimed to reveal urinary markers to proof uptake of 4F-MDMB-BINACA and differentiate from structurally similar SCs with valine and tert-leucine moieties like 5F-ADB, 5F-AB-PINACA and 5F-AMB-PINACA. In addition, basic pharmacological data on CB1 receptor binding and activation were produced.

Methods
Phase-I metabolites detected in human urine specimens were confirmed by a pHLM (pooled human liver microsomes) assay. Seized materials and test-purchased ‘legal high’ products and ‘research chemicals’ were analyzed by GC-MS and LC-QToF-MS. Human urine specimens and pHLM assay extracts were measured with LC-ESI-MS/MS and confirmed by LC-QToF-MS. Pharmacological data was obtained with a competitive ligand binding assay using [3H]-CP-55,940 and a receptor activation assay with [35S]-GTP at the human cannabinoid receptor 1 (hCB1).

Results
In January 2019, the Institute of Legal Medicine in Erlangen (Germany) identified 4F-MDMB-BINACA in three different, unlabeled herbal blends. During the same time period the described SC was identified in a research chemical purchased online with a purity of approx. 93%. Analysis of the phase-I metabolism of 4F-MDMB-BINACA in human urine specimens led to the detection of 13 metabolites. The identified metabolites are explicable by the following biotransformations: cleavage of the methyl ester, hydrolytic defluorination of the alkyl chain, oxidative defluorination to butanoic acid, hydroxylation of aromatic and alkyl structures, dehydrogenation and combinations of these metabolic pathways. Between August 2018 and March 2019, 57 urine samples have been tested positive for 4F-MDMB-BINACA metabolites. In total, 4140 urine samples were analyzed during that time period with a positive rate for SCs of 23.6% (n=977). The binding affinity of 4F-MDMB-BINACA at hCB1 was measured with a Ki value of 0.32 ± 0.18 nM. Its pharmacological potency as an agonist at hCB1 was proven by an activation assay.

Discussion
The most abundant metabolite of 4F-MDMB-BINACA as well as 5F-AMB-PINACA and 5F-AB-PINACA is the metabolite built by the hydrolysis of the ester or amide function. These isomeric hydrolysis products elute nearly at the same time under the chosen chromatographic conditions of the routine screening method for metabolites of SC in urine specimens. Differentiation was achieved by detection of the product ions still containing the fluoroalkyl chain but not the tert-leucine or valine residue (m/z 219, m/z 233). 4F-MDMB-BINACA shows relatively high affinity to the hCB1 receptor. Pharmacological potency of 4F-MDMB-BINACA as an agonist at hCB1 receptor was proven by an activation assay.

Conclusions
Investigation of phase-I biotransformation of 4F-MDMB-BINACA led to metabolites formed by ester hydrolysis and dehydrogenation as reliable urinary markers for proof of consumption. Differentiation between uptake of 4F-MDMB-BINACA and uptake of structurally similar, even isomeric SCs can be achieved by choosing specific diagnostic product ions (m/z 219 vs. m/z 233). 4F-MDMB-BINACA was firstly reported to the EDND (European information system and data base on new drugs) in November 2018. The first urine sample retrospectively detected positive for the substance was collected in August 2018. Since then 57 urine samples were found positive for 4F-MDMB-BINACA at the Institute of Forensic Medicine in Freiburg (Germany). Pharmacological activity at the hCB1 receptor was confirmed by a competitive ligand binding and a receptor activation assay. Since 4F-MDMB-BINACA is based on an indazole core structure and also fulfils the other requirements of the German NpSG, it is prohibited in Germany. Therefore, the motivation for the market introduction was not the circumvention of legal regulations, but rather the undermining of drug tests performed in correctional facilities or other environments where abstinence is demanded.
Abstract ID 366

Fentanyl and Analogs: A Study of US Controlled Substances Act (CSA) Scheduling and Trends of Positive Results in Two Testing Populations.

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Aim

The widespread epidemic of drugs marketed as free of the criminal consequences of possession and ingestion or “legal highs” was, for years, largely related to compounds that mimicked the effects of THC, MDMA and amphetamine. Then in 2013, acetyl fentanyl was first identified in the US and a wave of mu-opioid receptor agonists ensued, resulting in an increase in opioid-related deaths and imperiling traffic safety. On February 7, 2018, the DEA placed all fentanyl-related substances not yet listed into Schedule I of the CSA. anecdotally, agencies soon noticed a decrease in fentanyl analog detections and an increase in fentanyl detections. The study aim was to investigate if scheduling laws fostered a change in fentanyl and analog prevalence in two testing populations.

Methods

Both accredited laboratories use comprehensive, validated, multi-faceted screening procedures to detect and quantify fentanyl and fentanyl-related compounds. These procedures include general unknown screening by mass spectrometry and targeted screening by immunoassay and LC-MS/MS and/or LC-HRMS. The NC-OCME-MEIS was queried for all toxicology results in death investigations for the period January 1, 2016 through September 30, 2018. A total of 153,553 laboratory results were recorded for the study period with 55,729, 57,698, and 40,126 for 2016, 2017 and 2018 (9-months), respectively. Likewise, the PBSO-CL database was queried for all toxicology results in DUI, DUID, and DFC cases investigated in Palm Beach County. Data related to these crimes is collected in a database to study trends and inform public health and safety policy.

Results

According to the NC-OCME data, the number of analogs detected peaked in August 2017 with 126 and declined to less than five in September 2018. The PBSO-CL data exhibited a similar pattern with a peak of 19 in June of 2017 and a sharp decrease in 2018 with zero cases in September. However, fentanyl cases exhibited a steady increase over the study period peaking in June 2018 with 134 cases for the NC-OCME. But remained steady for 2016 and 2017 with 55 and 59 cases, respectively and 59 documented through the first 9-months of 2018 for the PBSO-CL. Fentanyl administrations from hospital settings were not detected in the PBSO-CL data set but were not removed from the NC-OCME data set as the query did not support this action. However, previous research on fentanyl detection rates by the NC-OCME supports the conclusion that fentanyl detected as a result of hospital administration are sufficiently rare in this test population not to affect the conclusions.

Discussion

Conclusions

The detection of fentanyl in casework in two study populations increased despite being a scheduled substance for many years, whereas, fentanyl analogs decreased during the same period. Prior to DEA actions, fentanyl analogs were considered to be “legal highs” and a decreased risk of legal consequences may have played a role in their distribution and use. Scheduling of fentanyl analogs appeared to foster a decrease in the incidence of these dangerous compounds.
Abstract ID 380
Ten Years of Experience with Synthetic Cannabinoids in the United States.

Melissa Fogarty
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Aim
Synthetic Cannabinoid Receptor Agonists (SCRA’s) were created by academic researchers in the 1980s and 1990s to elicit therapeutic cannabinoid-like effects such as anti-emetic and appetite stimulant effects. Researchers created over 600 different synthetic cannabinoid compounds. Although never commercialized, the compounds emerged in the street drug supply in 2008. JWH-018 was the first synthetic cannabinoid detected in illicit drug markets in Germany and Austria, and quickly spread worldwide. Subsequently successive waves of synthetic cannabinoid variants appeared in synthetic drug markets and online, and have remained popular ever since, and are still among the most commonly reported new psychoactive substances in 2019. In Europe, the European Monitoring Center for Drugs and Drug Addiction (EMCDDA) has reported 180 different synthetic cannabinoids between 2008-2019.

This presentation will focus on the changing landscape of synthetic cannabinoids in forensic casework within the United States over the ten-year period between 2010 and 2019 and describe a sentinel program for identifying and reporting on SCRA trends. We describe various findings from the analysis of seized material and toxicological samples, to create a timely approach to the identification of newly emergent SCRA’s and for monitoring trends in their popularity.

Methods
Since 2010, NMS Labs (Willow Grove, PA, US) and the Center for Forensic Science Research and Education (CFSRE) at the Fredric Rieders Family Foundation (Willow Grove, PA, US) have cooperatively tested for defined panels of synthetic cannabinoids, and collected and data-mined High Resolution Mass Spectrometry (HRMS) data from forensic toxicology specimens from human performance and death investigation casework. NMS Labs uses a liquid chromatography tandem mass spectrometry (LCMSMS) defined scope SCRA testing panel, which is regularly updated in line with changes in drug trends identified through various drug intelligence monitoring channels. In 2018, CFSRE began testing for synthetic cannabinoids by liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) using an extensive database containing more than 250 synthetic cannabinoid related compounds and their metabolites, with the goal of detecting newly emergent synthetic cannabinoids not included in the routine scope. Using this approach, CFSRE retested de-identified discarded biological sample extracts, previously screened for the defined SCRA panel, for an ever-growing expanded scope. Biological specimens from other forensically relevant populations including prisons and clinical populations were also tested. The toxicology data were compared to data from seized drug casework from street level drug seizures, as well as drugs seized at ports of entry into the United States.

Results
Data collected since 2010 depicts the rapid turnover of the SCRA drug market. The data show cyclic drug popularity synchronized with changes in legislation and scheduling. Examples include, a change in the market in 2011 when AM-2201 and JWH-122 were placed in Schedule I. XLR-11 immediately appeared in sample populations. More recently, the decline of FUB-AMB (MMB-FUBINACA) became apparent after scheduling in 2017. 5F-ADB (5F-MDMB-PINACA), which was also scheduled in 2017, did not however follow this trend, with positivity in biological samples continuing to rise a year after the scheduling took effect. As 5F-ADB positivity began to decline in 2018, a new synthetic cannabinoid, 5F-MDMB-PICA, started to replace it.

Since mid-2018, six new synthetic cannabinoids have been identified in biological extracts, including 5F-MDMB-PICA, 5Cl-AKB-48, 4-cyano-CUMYL-BINACA, 5F-EDMB-PINACA, 4F-MDMB-BINACA and APP-BINACA (www.npsdiscovery.org). All six of these emergent compounds identified from sample mining procedures were rapidly reported to the forensic science community for monitoring their appearance in toxicological casework.

This data has proven effective in assisting with the investigation of adverse drug reaction outbreaks. In July 2018, an outbreak of overdoses linked to heroin/fentanyl packets adulterated with 5F-ADB, signified our first encounter with synthetic cannabinoid-laced heroin/fentanyl products.

Discussion
SCRA’s continue to be important substances in death investigation and human performance casework, and the substances in circulation still turn over with some frequency. A combined approach of gathering information from seized drug casework, routine, intelligence-led updating of the analytical scope for SCRA toxicological panels, and datamining of HRMS data archives is a comprehensive approach to keeping up with this challenging drug class.

Conclusions
Since 2010, NMS Labs (Willow Grove, PA, US) and the Center for Forensic Science Research and Education (CFSRE) at the Fredric Rieders Family Foundation (Willow Grove, PA, US) have cooperatively tested for defined panels of synthetic cannabinoids, and collected and data-mined High Resolution Mass Spectrometry (HRMS) data from forensic toxicology specimens from human performance and death investigation casework. NMS Labs uses a liquid chromatography tandem mass spectrometry (LCMSMS) defined scope SCRA testing panel, which is regularly updated in line with changes in drug trends identified through various drug intelligence monitoring channels. In 2018, CFSRE began testing for synthetic cannabinoids by liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) using an extensive database containing more than 250 synthetic cannabinoid related compounds and their metabolites, with the goal of detecting newly emergent synthetic cannabinoids not included in the routine scope. Using this approach, CFSRE retested de-identified discarded biological sample extracts, previously screened for the defined SCRA panel, for an ever-growing expanded scope. Biological specimens from other forensically relevant populations including prisons and clinical populations were also tested. The toxicology data were compared to data from seized drug casework from street level drug seizures, as well as drugs seized at ports of entry into the United States.
Abstract ID 397

Tramadol distribution in hair after a single dose – a controlled study over four months.

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Aim
The aim of this study was to establish reference levels of tramadol and its main metabolites in hair after administration of a single dose of tramadol, and to investigate drug distribution by analyzing short segments of hair samples taken at several different sampling times.

Methods
An oral dose of either 50 or 100 mg of tramadol was administered to 17 healthy volunteers. Hair samples were collected prior to drug administration and 14, 30, 60 and 120 days after intake. Each sample was segmented in several 0.5 cm segments depending on sampling time and washed once with isopropanol and twice with water. Tramadol and its metabolites N-desmethyltramadol (NDMT), and O-desmethyltramadol (ODMT) were extracted from 10 mg pulverized hair incubated for 18 hours at 37°C in a media containing methanol, acetonitrile and 2 mM ammonium formate (25:29:46, pH 5.3). A previously validated and published UHPLC—MS/MS method for among others tramadol and ODMT was used to quantify the analytes (Wang et al. 2017, Drug Test. Anal. 9, 1137-1151). Supplemental validation for NDMT was performed. The LLOQ was 0.001 ng/mg for all analytes.

Results
A total of 84 hair samples were obtained from 17 subjects as for one subject the sample 14 days after was lacking and two subjects had too short hair to analyze the late sampling periods. One subject had black hair, 13 brown hair, one red hair and two blond hair. Tramadol and metabolites were detected in all relevant segments of each sampling time when assuming a growth rate of 1 cm/month, however some neighboring segments also showed positive results. The highest concentrations were observed in the proximal segment (0 - 0.5 cm) in the sample 14 day after intake of all subjects; 0.061-0.95 ng tramadol per mg hair, 0.012-0.86 ng NDMT per mg hair, and 0.009-0.17 ng ODMT per mg hair (n=16). Generally, the tramadol concentration was higher than the metabolites with a mean value of 0.26 for both metabolite/drug ratios and ranges of 0.12-0.40 for NDMT/tramadol ratios and 0.15-0.46 ODMT/tramadol ratios for segments of the highest concentrations of tramadol, respectively. However, one subject from the 100 mg dosage group deviated as generally higher amounts of NDMT than tramadol was observed. Some sweat contamination with tramadol only was observed in many segments, but in two subjects a constant level of tramadol were seen in all investigated segments for the samples 14 days after and also for one of them 30 days after intake. No analytes were detected in the last aqueous wash fractions of all analyzed segments. We also observed a wash out effect over time as the sum of all segments concentrations of each sampling period decreased from 30 days to 120 days. There was no difference between the two dosis groups, instead a great inter-individual variation was seen. An example is the subject with black hair who had the highest tramadol concentrations in hair although the low 50 mg dose was applied.

Discussion

Conclusions
Reference levels of tramadol and its main metabolites in hair were established following a single dose of tramadol. We also showed that analysis of short segments improved the determination of the exposure time after a single intake of tramadol, and that the highest concentrations of tramadol of each sampling period were found in the expected segment(s) although in the late sampling period(s) the distribution were more skewed. Finally, it was observed that collection of hair samples 14 to 30 days after an exposure provides the best conditions to detect and interpret the results.
Abstract ID 398
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Aim
In the casework of the Institute of Legal Medicine Munich the synthetic cannabinoid (SC) 5F-ADB (also known as 5F-MDMB-PINACA) unexpectedly turned out to be present in several cases with knock-outs. The aim of this study was to summarize the cases of drug-facilitated crimes (DFC) associated with the consumption of 5F-ADB, to determine typical symptoms and a possible correlation to measured drug concentrations in plasma.

Methods
The database of the Institute of Legal Medicine in Munich including cases in the timeframe from January 2014 to March 2019 was searched for 5F-ADB positive cases. The obtained cases were then filtered for drug-facilitated sexual assaults (DFSA) and robberies (DFR). Finally, these cases were further examined for information about victims, circumstances, described symptoms, and time intervals between incident and sample collection. Quantitative sample analysis was performed at the Institute of Forensic Medicine in Freiburg using liquid chromatography tandem mass spectrometry (LC-ESI-MS/MS) after liquid-liquid extraction of the samples.

Results
Data search obtained ten 5F-ADB positive cases from February 2016 to May 2018 associated with DFC. The intake of 5F-ADB could either be proven directly in blood (7) or via metabolites (corresponding carboxylic acid and/or 5-hydroxylated 5F-ADB) in urine (3). Crimes could be categorized in drug-facilitated sexual assault (7, victims all female) and robbery (3, victims all male). Some victims reported that they thought they smoked a marijuana joint offered by the perpetrator(s) others consumed synthetic cannabinoids (“herbal highs”) voluntarily but did not expect the strong effects. The age of the victims ranged from 15 to 41 years (median 17). Additionally, in all cases a comprehensive toxicological analysis was performed. Besides 5F-ADB, in four cases ethanol (3) or other central depressant drugs (1) could be detected. In six cases, 5F-ADB was the only substance detected with potential central depressant effects. The most prevalent described effect after smoking a cigarette/joint containing 5F-ADB was a memory lapse most probably due to a loss of consciousness. In addition, victims reported suffering from nausea, vomiting, amnesia and some kind of cataplexy. As the samples were usually delivered by the police no further clinical data were available. Plasma concentrations ranged from <0.1 µg/L to 0.58 µg/L. Time intervals between incident and sample collection ranged from 90 minutes to 11 hours when detected in blood. In cases where only metabolites of 5F-ADB could be detected in urine, time intervals were up to two days.

Discussion
The intake of 5F-ADB could be proven by detection in urine or blood in ten DFC cases. The measured plasma concentrations should be interpreted carefully because of the ester structure of 5F-ADB. Esters are rapidly converted to the corresponding carboxylic acid either by metabolic steps or by degradation after sample collection. 5F-ADB carboxylic acid was not included in the analysis performed. Thus, correlation of plasma concentrations and described effects was not possible.

Conclusions
The study summarized a series of ten 5F-ADB positive cases identified from February 2016 to May 2018 in Munich, Germany. The cases show that 5F-ADB is a very potent synthetic cannabinoid which after consumption can lead to severe adverse effects such as sudden loss of consciousness. Therefore, 5F-ADB might be used in the context of drug facilitated sexual assault or robbery as an incapacitating drug.
Abstract ID 414
Baseline Endogenous GHB Concentrations in Hair.
Mark Miller
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Aim
Hair can often be a useful sample for testing in drug-facilitated crime (DFC) cases as it provides a longer detection window. Delay in reporting a DFC that involves GHB (gamma-hydroxybutyric acid) is complicated by its rapid metabolism and clearance from the body, as well as its endogenous presence. A study was undertaken to examine the baseline variation of GHB in hair to assist in distinguishing endogenous versus exogenous concentrations.

Methods
Using a synthetic hair matrix for calibrator samples, validation of a liquid chromatography tandem mass spectrometry (LC-MS/MS) quantitative method for GHB in human head hair was completed. The method was valid over a range of 0.4-12 ng/mg, and its LOD and LOQ were both experimentally estimated to be 0.4 ng/mg.

Results
After validation, the variation in endogenous GHB concentrations across multiple donors and locations in the vertex posterior region of the human head were evaluated. Results for 11 non-GHB users showed minimal variability (average 3.0% RSD) across the vertex posterior for hair samples taken from three different areas. There was also low variability (average 1.8% RSD) in repeat samples taken from the same location for 11 other non-users.

While many studies over the years have worked to resolve the challenges encountered when analyzing GHB in hair, few have had large populations. More than 2000 hair sample segments (1 cm) from 141 women and 73 men (all collected hair 3-12 cm in length) were analyzed. The range of endogenous GHB concentrations observed was <0.4-5.47 ng/mg and 97.5% of the segmental results were less than 2 ng/mg.

Discussion
A Kruskal-Wallis comparison of segmental medians in males and females indicates that these groups are different, with greater than 95% confidence. Additionally, female hair samples appeared to have a trend comprising higher endogenous GHB concentrations close to the scalp and with a net decrease of −0.2-0.3 ng/mg distally. Male hair samples displayed the opposite trend, with a net increase of −0.5-0.6 ng/mg moving towards the distal end of the hair shaft. However, there was minimal change between a donor’s adjacent hair segments, with 97.1% of adjacent segment differences within ±0.5 ng/mg, across the population.

Conclusions
Based on the wide concentration range detected in our population, it appears difficult to select an appropriate cut-off for differentiating endogenous GHB from exogenous without a large controlled dosing study. In the absence of a recommended cut-off, using adjacent segment concentration differences could be a strategy to assist in differentiating endogenous from exogenous GHB exposure.
Abstract ID 456
A Voice in the Wilderness: Unexpected Drugs Found in Unregulated Cannabidiol E-cigarette Products by the Unsuspecting Public.
Michelle Peace
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Aim
Electronic cigarettes (e-cigs) were developed as a method for nicotine delivery. The e-liquid formulations are made of a ratio of propylene glycol and vegetable glycerin and/or a pharmaceutical and/or herbal remedy plus, potentially, a flavoring agent. E-cigs have also been adopted by users to also inhale drugs other than nicotine (DOTN), including cannabidiol (CBD). CBD is a significant active ingredient of C. sativa and C. indica and has been purported to have anti-convulsant, anti-nociceptive, and anti-psychotic properties, and is a popular ingredient in e-liquids used in e-cigs. A single CBD formulation has been approved by the United States Food and Drug Administration for the treatment of two rare forms of epilepsy. The Farm Bill recently legalized hemp production in the United States, from which CBD can be extracted. If hemp contains more than 0.3% THC, the plant is considered a non-hemp product. A federally unregulated consumer-driven market of cannabinoid products, in combination with the loosely regulated e-cig market, has created public health and public safety challenges with toxicological emergencies. The purpose of this research was to identify unlabeled psychoactive compounds in CBD e-liquids submitted by persons claiming unexpected untoward effects.

Methods
Eight samples were received from individuals across the United States who had purchased CBD e-liquid products from either online or retail outlets for therapeutic purposes. A 10 µL aliquot of liquid samples and 10 mg aliquot of wax samples were diluted to 1 mL with methanol and analyzed using a Shimadzu QP2020 GC-MS. When required, samples were diluted again 1:10, with methanol. In brief, a 1 µL aliquot was injected onto a Agilent DB-5MS narrow-bore capillary column (15 m X 0.25 mm, 0.25 µm film thickness). The injection port and transfer line temperatures were 275 ºC and 280 ºC, respectively. The GC oven was programmed to an initial temperature of 70 ºC followed by a 15 ºC/min ramp to a final temperature of 300 ºC held for 7 min with a total run time of 27 min. Analytes were identified using the SWGTOX library and published reference methods. Compounds were confirmed by comparing retention times and full scans using certified reference materials, where available.

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

Discussion
Most accounts were made by persons who claimed CBD use was for therapeutic benefit as opposed to recreational use. The dose consumed by each person is unknown, even if an account of consumption was provided, since the volume of aerosol inhaled is unknown. One account was a teenager who was described as having become addicted to CBD. Reported use of these products led to statements such as “I have not been able to leave my apartment for four days”, “What happened next I can only describe as the situation rapidly devolving into the scariest night of my life – I felt like I was dissociating from reality”, “one of the times I confronted [my son] in his room pale and glassy eyed”, and “have you...tested other popular so called ‘CBD-juice brands such as...”, and “a 79 year old grandmother just wanted to be pain-free, but she had severe hallucinations”. All persons were surprised by the effects but did not know what action could be taken to report the e-liquid companies or distributors. Several were concerned that any attention would have adverse recourse, both personally and professionally.

Conclusions
Analysis of the eight samples revealed a number of unexpected novel psychoactive compounds that accounted for the untoward effects the individuals described. Drug concentrations of each sample are not indicative of the doses consumed by the individuals, which then elicited untoward adverse effects. The federally unregulated cannabinoid market, combined with an e-cig market with loose quality assurance requirements, has created a significant public health and public safety problem in the United States. As such, it is critical for the forensic toxicology community to publish findings from casework which could demonstrate the breadth of the problem and provide a warning with wide implications for public safety.
Abstract ID 515
Wastewater-based epidemiology combined with forensic toxicological information: The approach exemplified by cocaine and methamphetamine use in Finland.
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Aim
Wastewater-based epidemiology (WBE) has recently become one of the most rapidly-evolving disciplines engaged in the measurement of population-level illicit drug use. While WBE approach does not provide any information on users at individual level, combining WBE with forensic toxicological casework data offers important information on, e.g., user demographics and polysubstance use. Toxicological data also implies how increased population-level drug use may increase harmful societal phenomena, such as driving under influence of drugs (DUID). This study shows how local and wide-scale national studies based on WBE methodology can efficiently give early-warning type of information on drug use and its trends, and linked to traditional toxicological data to provide novel and comprehensive information for various stakeholders including law enforcement, health authorities, practitioners, decision makers, and other stakeholders.

Methods
Wastewater analysis: 24-h wastewater influent composite samples (n=109, all Sunday 07:00 to Monday 07:00) were collected from Helsinki wastewater treatment plant (WWTP) serving approximately 855,000 inhabitants during 04/2013-02/2019. Furthermore, several national sampling campaigns up to 24 WWTPs covering approximately 55 % of all Finnish population (3 million out of 5.5 million) during 2012-2018 were performed. All samples were analysed by validated UHPLC-MS/MS. Traffic cases: WBE studies have recently indicated major changes in methamphetamine and cocaine use in Finland. The prevalence of these analytes was therefore studied in detail in all suspected DUID cases in Finland (from 02/2013 to 12/2018). In addition, simultaneous use of other drugs (polysubstance use), as well age and sex of cocaine users, were recorded, to provide more detailed information on actual users. All cases were systematically analysed by the chromatographic mass spectrometric techniques.

Results
Both wastewater analysis data and suspected DUID cases confirm the significant rise of cocaine use during the last few years in Finland. Based on WBE, cocaine use is still highly concentrated in the capital area of Finland and there has been 5-6-fold increase in cocaine use at population-level during the last five years. During 2013 and 2014 benzoylecgonine (cocaine metabolite) concentrations were generally around 15-20 mg/1000 persons/day at the wastewater of Helsinki WWTP, while increasing during 2015 and 2016, then at the same level in 2017 than in 2016, again raised to the record-high level in 2018, and reaching close to 120 mg/1000 persons/day (on average) at the first half of the year in 2019. At the same time, cocaine-related DUID cases increased from 81 cases (1.8% of all suspected cases) in 2013 to 478 cases (5.5%) in 2018 at the national level. For methamphetamine, DUID cases in Finland increased from 366 (6.0%) in 2015 to 1625 (24.9%) in 2016 cases during one year. The earliest indications of sudden increase of methamphetamine use were already noticed in the beginning of 2016 based on WBE studies. In 2018, the use of methamphetamine was significantly lowered again in Finland, which is confirmed by both WBE and DUID studies.

Discussion
These results show in detail that the changes of population-level drug use can be very rapid both regionally and nationally. Furthermore, the results exemplify how population level increase in illicit drug use may also strongly affect to presence of these drugs at harmful events, such as driving under influence of drugs, and therefore findings at toxicological casework. WBE and DUID results strongly correlated with each other for both cocaine and methamphetamine use at the national level. Unfortunately, local DUID data was not available for more regional comparisons at the moment.

Conclusions
WBE approach offers objective, novel and nearly real-time estimates on drug use and its trends for various illicit drugs, both regionally and at national level. More comprehensive picture on drug use can be obtained when combining WBE information with traditional forensic toxicological indicators, as exemplified here with DUID data. Information gathered by this approach has been of great public interest, but also of significant practical value for various stakeholders and a basis of evaluation of current (illicit) drug situation in Finland.
Abstract ID 525
Toxicological Findings from Alleged Sexual Assault Victims in Native American Communities from 2011 to 2018.
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Aim
Self-governing Native American communities throughout the United States are termed as “Indian Country”. Indian Country spans thousands of rural areas, towns, and cities where Native Americans live. Major crimes committed by or to a Native American citizen fall under the jurisdiction of the United States Government. As such, the FBI assists in the investigation of allegations of sexual assault that occur in Indian Country and the Laboratory Division of the FBI conducts many of the forensic analyses on evidence in these cases. The aim of this study was to evaluate the toxicological findings from alleged sexual assault victims in Native American communities for the time period of 2011 to 2018.

Methods
Records of suspected sexual assault cases from 2011 to 2018 that occurred in Native American jurisdictions and were submitted to the Toxicology Group of the FBI Laboratory’s Chemistry Unit were reviewed. 78 such cases were received in which the law enforcement investigation suggested that alcohol and/or sedative drugs may have played a role, thereby allowing them to be subclassified as a potential drug-facilitated sexual assault.

Results
The alleged victims had specimens collected within 24 hours of the alleged assault in 58% of the cases; 1 to 3 days in 27% of the cases; and more than 3 days in 1 case. Specimen collection times were not provided in the remaining cases.

Both blood and urine were collected in 45% of the cases, compared to only blood or urine in 40% and 13%, respectively. In one case, a hair sample was the only specimen submitted for testing.

One or more drugs capable of causing CNS depression were identified in 66% of the cases. The most-prevalent toxicological finding was ethanol identified in 49% of the cases. Other prevalent findings of CNS depressants included opioids (18%), benzodiazepines (12%), sedative antihistamines (11%), and barbiturates/other anticonvulsants (11%). Less common, yet potentially relevant findings included sedative antidepressants, antipsychotic agents, and muscle relaxants. Other toxicological findings included cannabinoids in 43% of the cases and amphetamine and/or methamphetamine in 28% of the cases.

Discussion
The findings of this study are consistent with the work of others worldwide and support the notion that ethanol is the most-prevalent finding in cases of drug-facilitated sexual assault. This is not surprising, as there is a high prevalence of alcohol use among Native Americans. Likewise, the other drug prevalence findings in this study mimic the recreational and prescription medications that are popular in the United States, as opposed to the so-called “date rape” drugs popularized by media. This may suggest that these alleged sexual assaults may be more opportunistic in nature, as opposed to the perpetrator surreptitiously administering an incapacitating agent.

Conclusions
A recent study found that 56% of Native American women have experienced sexual violence. Alcohol and drug use are often associated with these assaults. While the results of toxicological analysis alone cannot prove or disprove a drug-facilitated sexual assault has occurred, the findings of one or more CNS depressant drugs in an alleged victim may provide evidence of their inability to consent to a sexual act.
Abstract ID 544
Screening of New Psychoactive Substances, THC and cocaine in urine samples obtained at two music festivals in the metropolitan area.

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Aim
We present the results of two studies of effluent samples that were obtained in the context of two electronic music parties. The aim was to generate accurate information on the circulation in the Uruguayan territory of New Psychoactive Substances and other drugs of abuse that are consumed in these events.

Methods
During the parties a modified chemical bath was used. This chemical bath allowed to collect pools of urines every five persons who entered in the bathroom. An aliquot of these pools was removed to be later analyzed in the laboratory. During the study no survey or identification of the people who used the bathroom was done.

A total of 28 samples of the first party (2017) and 25 samples of the second party (2018) were analyzed by: immunological tests for the detection of: Opiates, Fentanyl, Synthetic Cannabinoids, Catinones, THC, Cocaine and its metabolites and 6-MAM and by GC/MS. The samples were processed by four different methods, in different physicochemical conditions to make possible, in each case, the extraction of drugs of diverse nature contemplating the neutral, basic, acid substances and those excreted as metabolites and/or conjugated. Each of the extractions were then analyzed by Gas Chromatography coupled to Mass Spectrometry (GC/MS).

Results
As remarkable results in the first party was found: Synthetic Cannabinoids in 11% of the samples, Catinones in 52%, Cocaine/PBC/Cocaethylene and metabolites in 82%, Ketamine in 11%, Levamisol in 7%, LSD/LAMPA in 4%, MDMA in 71%, THC in 100%, and 2.5B-NBOMEs or bk-DMBDB were not detected. In the second party was found: 6-Acetylmorphine (Heroin) in 8% of the samples, indicating the appearance of consumption of this substance, Catinones in 52%, Cocaine/PBC/Cocaethylene and metabolites in 96%, LSD/LAMPA in 8%, MDMA in 64%, THC in 96%, 2.5B-NBOMEs in 4% and bk-DMBDB in 4% of the samples analyzed. Levamisole, Fentanyl and Ketamine were not detected.

Discussion
The tracking of the profile of consumption in electronic parties has allowed to detect the consumption of substances that had no history in the country of being consumed or either been seized. In this work we also compare the findings obtained between the two parties, which allowed to see how it is a dynamic system in the consumption of new psychoactive substances and a quite stable consumption of cannabis and cocaine.

In the particular case of the cathinone bk-DMBDB, it allowed us to detect it in the country six months before we have the first serious intoxicated case with it.

Conclusions
This work provides accurate and valuable information of the substances that were used in these parties. Some of the substances found were not seized and some of them were detected in intoxication cases afterwards. The information collected was consider very important by policy maker in drug of abuse and damage reduction.
Abstract ID 14

Long-term abuse of clenbuterol and stanozolol: post mortem investigations.

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Aim

The authors present here a rare fatal case involving clenbuterol, abused by a 61-year old man for bodybuilding purposes. Complete (autopsy, histology and toxicology) investigations will allow to document and discuss the case.

Methods

The body of a 61-year old man was found at his home by his wife, lying on the floor, near the bathroom, around mid-night. He was known training for body building, using anabolic steroids. Police investigations revealed the presence of 2 types of tablets at home, one supposed to contain clenbuterol (0.040 mg), the other stanozolol (10 mg).

Complete investigations, including autopsy, histology tests and toxicological analyses were performed to document the case.

Results

Testing the tablets revealed different dosages from what was expected, i.e. 0.073 and 11.5 mg/tablet, for clenbuterol and stanozolol, respectively.

External body examination and autopsy, performed the next day, revealed generalized organ congestion and the lack of any traumatic injury (confirmed by radiology). Cardiomegaly, with a heart weighting 692 g, was obvious.

Anatomic pathology tests did not evidence malformations but atheromatous plaque was identified in the coronaries during complete histology investigations.

Femoral blood, urine, bile, gastric contents and 2 strands of hair (6 cm) were collected for toxicology. These specimens were submitted to standard analyses, but also to a specific LC-MS/MS method for clenbuterol and stanozolol testing. Clenbuterol was identified in all the tissues, including femoral blood (1.1 ng/mL), urine (7.2 ng/mL), bile (2.4 ng/mL), gastric content (3.2 ng/mL) and hair (23 pg/mg). Stanozolol only tested positive in hair (11 pg/mg).

All other analyses were negative, including blood alcohol and drugs of abuse.

Discussion

The pathologists concluded to cardiac insufficiency with support of cardiomegaly, in a context involving repetitive abuse of anabolic drugs. This case indicates that more attention should be paid to clenbuterol, a drug widely used as a stimulant by people who want to lose weight, athletes and body building practitioners.

The combination of clenbuterol and stanozolol can be considered as a synergetic preparation. In this case, it was of paramount importance to test for both drugs in hair to document the case and the possible side-effects, responsible for enhancing the fatal process. Only clenbuterol was detectable in blood at low concentration and stanozolol was not identified. The use of hair allowed to have a parallel between heart disease with long-term anabolic drugs abuse.

Conclusions

Toxicologists and forensic pathologists should be aware of possible abuse of clenbuterol as a product proposed to lose weight or to enhance athletic performance, even in subjects older than 60. The possibility of cardiac insufficiency in a subject suffering from silent cardiomegaly in the presence of occlusive coronary artery disease must be documented by complete autopsy and anatomic pathology tests.

A comprehensive toxicological screening, targeting all the performance-enhancing drugs is of paramount importance, as clenbuterol and anabolic steroids are seldom tested and blood concentrations are generally very low. Even abused alone, clenbuterol or stanozolol will increase the risk of cardiovascular disease. Therefore, monitoring all the drugs with a cardiac tropism, even indirectly, is of importance when the death is unexpected, particularly in the bodybuilders population or subjects who want to lose weight.

This fatal case demonstrates the absolute need of collaboration between the active investigators, including Police, forensic pathologists (autopsy + anatomic pathology tests) and toxicologists. This is the price to pay as only very few case reports dealing with the combination clenbuterol and steroids are available in the literature, although these drugs are abused by millions of subjects.
Abstract ID 32
Testing for an anabolic androgenic steroid, stanozolol, in hair collected from 5 different anatomical regions.

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Aim
Stanozolol is a prohibited substance by the World Anti-Doping Agency, in-and-out of competition. This product is classified S1 as an anabolic androgenic steroid. In 2017, it represents 20 % of the total adverse analytical findings for the S1 class. An athlete challenged the result from an in-competition test which returned with an adverse analytical finding for stanozolol and his lawyer asked us to analyze several hair specimens simultaneously collected from 5 different anatomical regions, i.e. head hair (about 4 cm), arm hair, leg hair, pubic hair and under arm hair.

Methods
Given the curvy nature of the head hair, there was no attempt to do segmentation. Blank hair for method validation was obtained from laboratory staff. After decontamination with dichloromethane, hair specimens were weighted and incubated in 1 mL NaOH 1 M during 15 min at 95°C, in the presence of 300 pg stanozolol-d3 used as internal standard. After cooling, 5 mL ether were added, and, after centrifugation, the organic phase was dried and was reconstituted with 50 µL of methanol. A specific method was developed by ultra-high-performance liquid chromatography system coupled to a tandem mass spectrometry using an Acquity UPLC HSS C18 column (150 mm x 2.1 mm, i.d. 1.8 µm particle size). A gradient elution was performed using 1.0 mM ammonium acetate in water (eluent A) and 0.1 % formic acid in acetonitrile (eluent B) at flow rate of 0.3 mL/min. Analysis time was 6.0 min and the following gradient pattern (eluent A) was used: 0 min, 95%; 2.00 min, 95%; 4.00 min, 0 %; 4.10 min, 0%; 4.30 min, 95%; 6.00 min, 95%. Stanozolol and stanozolol-d3 were eluted in 4.9 min. Cone voltage and collision energy were adjusted to optimize the signal of the 2 most abundant product ions of stanozolol: m/z 329.3>81.1 (CV: 86 , CE: 44) and for stanozolol-d3: m/z 332.3>81.1 (CV: 80 , CE: 52).

Results
Linearity was observed for concentrations ranging from 5 pg/mg to 10 ng/mg with a correlation R² of 0.9981. The lower limit of quantification was 5 pg/mg. The CV’s of repeatability and matrix effect were inferior than 20%. Concentrations of stanozolol in head hair, pubic hair, arm hair, leg hair and under arm hair were 73 pg/mg, 454 pg/mg, 238 pg/mg, 244 pg/mg and 7.1 ng/mg, respectively.

Discussion
The concentration of stanozolol in head hair is in accordance with data published in the scientific literature (2 – 180 pg/mg in human head hair). When comparing our data, body hair concentrations were higher than the concentration found in head hair. These results seem to evidence that stanozolol incorporates better into body hair than head hair. The very high concentrations in axillary hair (7.1 ng/mg) and in pubic hair (454 pg/mg) may be explained by contamination from sweat and urine, respectively. It is interesting to note that the concentrations in arm and leg hair are similar, which is consistent with close growing rates.

Conclusions
Stanozolol is a basic drug which incorporates well into hair. This steroid is the only one that has a nitrogen atom in its chemical structure. The simultaneous positive concentrations in different hair types confirm the adverse analytical finding in urine of the top athlete. In case there is no head hair, body hair could be a good complement to document an adverse analytical finding. For the first time, a doping agent was simultaneously tested in hair collected from 5 different anatomical regions from the same subject, with a large distribution of concentrations, due to anatomical variations.
No Pain No Gain – Influence of Pain Killers on the Anabolic Steroid Profile.

Anna Stoll

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Aim

Anabolic androgenic steroids (AAS) are prohibited in sports as performance enhancing drugs (class S1 in the list of prohibited substances issued by the World Anti-Doping Agency, WADA). To detect the misuse of those substances, among other methods, WADA accredited laboratories monitor the urinary steroid profile (amount and ratio of endogenous steroids, their precursors and metabolites) in urine samples collected from the athletes. As the steroid metabolism is very complex and many enzymes are involved, it is very likely that selected non prohibited drugs may influence steroid metabolite excretion. One important group of steroid metabolizing enzymes are the aldo keto reductases (AKRs). Byrns et al demonstrated in vitro that NSAIDs (non-steroidal anti-inflammatory drugs) inhibit AKRs [Byrns MC, et al. Biochemical pharmacology 75 (2008) 484–493].

The combination of facts mentioned above leads to the assumption that non prohibited NSAIDs interfere with the steroid metabolism and may therefore lead to deviating reporting in doping control. As NSAIDs are very frequently used drugs in sports, this work aims to investigate the influence of NSAID intake on the urinary steroid profile.

Methods

Model steroids were used for in vitro investigation on biotransformations catalyzed by 17β hydroxysteroid-dehydrogenase (17β-HSD) or 3α-HSD (AKR1C3 and AKR1C2). To identify the products of the AKR-catalyzed reactions, relevant incubations were analyzed by GC/MS. Kinetic and inhibitory studies were performed exemplarily using androstenedione (4 AED) and 5α dihydrotestosterone (5αDHT) as substrates of AKR1C3 and AKR1C2, respectively. Incubations were performed and the depletion of co factor (NADPH) was determined in real time by fluorescence-intensity measurements. Furthermore, measurements on a GC/Q-ToF instrument were carried out to confirm findings from fluorescence intensity measurements and to confirm product formation.

To investigate the relevance in vivo, urine-samples of volunteers prior, during and post administration of Ibuprofen and Indomethacin were analyzed to monitor the steroid profile.

Results

Product formation of AKR-catalyzed reactions could be shown for different relevant substrates. Corresponding 17β-hydroxy- and 17-oxo-steroids (or 3α-hydroxy- and 3-oxo-steroids) were detected in the incubation broth utilizing GC/MS analysis. Kinetic parameters like KM and Vmax were determined for the investigated reactions. Furthermore, the inhibitory extend of NSAIDs was investigated and inhibitory patterns were examined.

The findings form in vitro experiments were compared to findings from in vivo investigations. Changes of some steroid profile ratios were observed.

Discussion

AKR1C2 and AKR1C3 were chosen for investigation due to the key role of those two aldo-keto reductases in the human androgen metabolism. As mentioned above, alterations in male sex steroids are used as indicators for the misuse of anabolic steroids in sports. Therefore, especially their metabolic pathways with possible interactions are crucial to elucidate. The results obtained from in vitro experiments show that NSAIDs interfere with steroid metabolizing AKRs and thus influence steroid biotransformation.

Conclusions

The presented work illustrates the importance to consider co-administration of (non prohibited) drugs during anti-doping analysis. The intake of multiple substances is likely to lead to interfering effects. Divergent results in anti-doping analysis may therefore be observed and misinterpretation of analytical data may occur. The combination of in vitro and in vivo results suggest that the monitoring of NSAIDs may be useful in doping control analysis. Similar considerations may be appropriate for other fields of forensic applications.
Abstract ID  300
Discrimination of microbial produced boldenone from its exogenous administration – examples from equine doping control
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Aim
Boldenone (1-dehydrotestosterone) is an anabolic-androgenic steroid, which is available for veterinary purposes, but prohibited as a performance enhancer in sports. It is also endogenously produced in the horse, being present in testicular tissue, which most likely accounts for its urinary excretion in the uncastrated male. Other potential sources are microbial conversion in feed, the gastrointestinal tract or voided urine. Ideally, distinguishing exogenous administration of boldenone from putative microbial action would be shown by differences in the carbon isotope content of the steroid, as carried out in human anti-doping. However, this approach is less suitable for doping control in equine sports due to the horses’ diet. An alternative approach was therefore required to identify microbial activity. The aim was to identify potential biomarkers that may be formed in conjunction with boldenone because of microbial activity in urine and feed.

Methods
Experiments were conducted initially using equine faeces to identify suitable biomarkers. Faecal incubations were carried out at room temperature in the presence of deuterium and carbon isotope-labelled steroids (D3-testosterone, 13C3-androstenedione and D9-progesterone). Following the identification of the potential biomarkers, the application of these biomarkers for the identification of inappropriately stored urine samples was assessed by incubating urine samples at room temperature in the presence of the aforementioned isotope-labelled steroid precursors. Additionally, urine samples collected following an oral administration of contaminated feed, found to contain boldenone, boldienone and Δ1-progesterone, to horses were analysed. All the faecal and urine samples were analysed both underivatised and derivatised to increase the certainty of identification and enhance the detection capability using liquid chromatography tandem mass spectrometry (LC-MS/MS) and LC high resolution mass spectrometry (LC-HRMS).

Results
Faecal incubations in the presence of isotope-labelled precursors showed the formation of isotope-labelled boldenone and boldienone from testosterone and androstenedione, as well as the formation of Δ1-progesterone and boldienone from progesterone. Unlabelled forms of these steroids were also produced in unspiked faecal samples.

Subsequent analysis of 30 incubated urine samples showed Δ1-dehydrogenase activity in seven samples where isotope-labelled Δ1-progesterone was detected in conjunction with either isotope-labelled boldenone or boldienone.

The feed administrations resulted in the detection of boldenone sulphate, boldienone and C20-reduced metabolites of Δ1-progesterone in urine samples.

Discussion
Faecal incubations displayed high Δ1-dehydrogenase activity helping identification of suitable biomarkers. As a result, Δ1-progesterone (1-dehydroprogesterone) was identified. The targeted analysis of incubated urine samples showed that this could be a potential biomarker to distinguish the microbial production of boldenone in voided urine samples from its exogenous administration. For the detection of microbial conversions in feed samples, this biomarker was not as suitable as it was subjected to first pass metabolism. However, it was possible to detect its C20-reduced metabolite in these administration samples, suggesting its applicability as a biomarker.

Conclusions
These results describe an alternative approach to aid distinguishing the origin of suspicious boldenone findings in cases where carbon isotope measurement would not be possible, such as in equine urine. The detection of Δ1-progesterone in urine could potentially be used to demonstrate microbial activity in voided urine samples, whereas the presence of C20-reduced urinary metabolites demonstrates microbial activity in feed.
Abstract ID 302
Tracing back drug misuse – proper metabolite identification requires synthesis.

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Aim
Xenobiotics may undergo biotransformation during body passage. The extent strongly depends on the structure of a compound. Tracing back drug misuse therefore requires profound knowledge of the metabolism of a compound. State-of-the-art metabolite identification often utilizes high resolution mass spectrometry for structure proposal. However, even in combination with transfer of knowledge from related substance biotransformation, correct interpretation requires reference material for final structure assignment. Noteworthy, reference material is also required for quantitation or evaluation of the method performance characteristics. In the present study, proper structure confirmation was performed for one of the recently detected metabolites of the anabolic androgenic steroid chlorodehydromethyltestosterone (DHCMT), which is one of the most frequently detected compounds in nowadays doping control analysis.

Methods
Post administration urines of the anabolic androgenic steroid 4-chlorodehydromethyltestosterone (single dose of 5 mg) have been analyzed by GC-QQQ-MS (MRM) after hydrolysis and derivatization (per-TMS derivatives). For confirmation of a recently reported metabolite (Sobolevsky et al JSBMB 128 (2012) 121–127), reference material was chemically synthesized. Different diastereomeric compounds were obtained due to the synthetic route and separated by semi-preparative chromatography. Retention times and mass spectrometric characteristics were compared to correctly assign the metabolite’s structure. NMR analyses were performed for structure confirmation and stereochemical assignment.

Results
As reported by Sobolevsky et al. A-ring reduced metabolites with 17-hydroxymethyl-17-methyl-18-nor-13-ene structure were excreted after the administration of DHCMT. Due to the very low concentration their detection required GC-QQQ-MS after formation of per-TMS derivatives. Among others, a metabolite called M4 by Sobolevsky was detectable for about 5 days after the administration of one single dose. The comparison with the chemically synthesized reference allowed for assignment of “M4” to 4-chloro-17α-hydroxymethyl-18-nor-17β-methylandrosta-4,13-dien-3β-ol.

Discussion
In contrast to earlier proposals and expectations from the general knowledge of steroid metabolic pathways, the stereochemistry of M4 was found surprisingly different in stereocenters at C3 and C17 from the proposal of Sobolevsky. Only by comparison with in-house synthesized reference, it was possible to properly assign the correct diastereomeric composition. NMR structure confirmation of the synthesized reference material was possible, while NMR analysis of the excreted metabolite was hampered by the very low amount and the complexity of the urinary matrix.

Conclusions
In many forensic applications and even more specifically in doping control analysis the utilization of mass spectrometry for metabolite identification is mandatory due to the ultra-trace amounts to be detected. However, isomeric compounds often yield very similar mass spectra and proper structure assignment is only possible by joint comparison of MS and chromatographic data with authentical reference material. To avoid misinterpretation in-house synthesis is required, if reference material is not commercially available. Transfer of these experiences is also relevant for NPS.
Abstract ID 392
Performance-enhancing substances used by bodybuilders.
Jean Claude Alvarez
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Aim
The aim of this study was to identify and quantify active ingredients present in products purchased on Internet and on the street market by bodybuilders.

Methods
Seizures made in sports halls and sent to our forensic toxicological laboratory from January 2016 to July 2018 were included. A qualitative analysis was performed by GC-MS (DSQ, Thermo Fisher, Les Ulis, France), LC-DAD (Acquity, Waters, Guyancourt, France) and LC-HRMS (Q-Exactive, Thermo Fisher). Quantification was carried out on an Ultra High-Performance Liquid Chromatography system coupled to a tandem mass spectrometer (TSQ Quantiva, Thermo Fisher) with an HypersilGold column. Human Chorionic gonadotrophin (HCG) was quantified with a specific immune-enzymatic assay (Dimension EXL, Siemens, Germany). CJC-1295, a synthetic analogue of growth hormone-releasing hormone, was identified by LC-HRMS [1]. Active ingredient and concentration were then compared to those mentioned on the label.

Results
Sixty-two products were analyzed, 48 pharmaceuticals (P) and 14 dietary supplements (DS). All DS were labeled as vitamins or amino acid concentrates, and no anabolic or pharmaceutical substance was detected among them. Among P, 34 (71%) contained the compound mentioned on the label: 14 (29%) were found at the right dosage, 11 (23%) with a different dosage, 7 (15%) could not be quantified (standard not available at the moment of analysis) and 2 (4%) had no dosage indicated on the label. Twelve P (25%) contained another substance than that indicated on the label, and no substance was detected for 2 products (4%). Regarding the pharmacological class, there were 34 (71%) androgenic anabolic steroids (AAS), 3 phosphodiesterase-5 inhibitors (tadalafil (n=2), sildenafil (n=1)), 3 β-2 adrenergic agonists (clenbutérol (n=3)), 3 peptide hormones (HCG (n=2) and CJC-1295 (n=1)), 2 benzodiazepines (lormetazepam (n=2)), 1 antalgic (nefopam), 1 selective estrogen receptor modulator (tamoxifen), 1 photosensitizing agent (methoxsalen). Twenty-three AAS were intramuscular injectable oils (mainly esterified steroids), and 11 were tablets (17 α-alkylated steroids).

Discussion
Out of the 48 analyzed P, more than half did not contain the compound mentioned on the label or contained the right compound with a different dosage, highlighting that bodybuilders did not know exactly what they were using. AAS were the most found substances, and high variability in content and potency of these compounds could be harmful to health [2].


Conclusions
To our knowledge, this kind of study is realized for the first time in France. The use of performance-enhancing drugs is a health problem probably largely underestimated.
Voluntary or accidental doping? That's the problem.

Donata Favretto

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Abstract ID 471

Aim

When a WADA-accredited laboratory identifies a prohibited substance or its metabolites in a doping control sample, an Adverse Analytical Finding (AAF) is notified to the athlete. An AAF most often leads to an anti-doping rule violation, unless the athlete has a Therapeutic Use Exemption for that particular substance, and to a period of ineligibility. However, if an athlete or other person establishes in an individual case that he or she bears no fault or negligence, then the period of Ineligibility shall be eliminated. If he or she bears no significant fault or negligence, the period of Ineligibility may be reduced according to the degree of fault or negligence.

The aim of the presented work is to discuss the role of toxicologist and toxicology methods in cases where an athlete inadvertently ingests or is exposed to a prohibited substance, and needs to document his/her reduced or null degree of fault.

Some real cases will be presented involving AAFs for letrozole, an aromatase inhibitor, hydrochlorothiazide, a diuretic and masking agent, clostebol, a synthetic anabolic androgenic steroid, and for the use of exogenous testosterone esters.

Methods

For letrozole (approved for the post–surgery treatment of breast cancer and thought to increase testosterone levels) a study on the urinary excretion and incorporation into hair of single doses of letrozole was envisaged to demonstrate that an accidental ingestion of less than a tablet through contaminated food can produce levels of metabolite in urine comparable to those of an AAF (case 1). The urinary concentrations of letrozole and its metabolite M1 were determined in urine samples collected for nine days after the consumption of 2.5 or 1.25 mg of letrozole for 7 days. In the athlete’s 5 cm long hair no letrozole was detected. In the athlete’s hair, no letrozole was detected along the 20 cm long hair shaft. Hair analysis turned to discriminate letrozole repetitive use vs occasional/inadvertent administration. The athlete received a reduced period of ineligibility.

For HCTZ, ingestion of contaminated supplements produced urinary concentrations higher than the WADA reporting limits up to 24 hrs; in the hair, collected after 15 days, no HCTZ was found. In the athlete’s 5 cm long hair no HCTZ was detected. The plausibility of the observed urinary concentrations with the supplement ingestion yielded a reduced period of ineligibility.

No clostebol or clostebol acetate was found in the hair of case 3 (male, 5 cm long) and case 4 (woman, 30 cm long, segmented to cover different periods before and after the alleged event). The finding supported the accidental and occasional intake of the prohibited substance through an ointment (reduced period of ineligibility) or contaminated meat (the ineligibility was eliminated).

As to case 5, the absence of all testosterone esters and exogenous steroids in the athlete’s hair supported a single exposure with null doping efficacy, but the athlete was deemed ineligible for the maximum period.

Results

Regular use of letrozole may result in accumulation of letrozole and its M1 metabolite. In hair collected after single dosages, concentrations of 30-60 pg/mg are detected in the segment corresponding to the day/week of administration; in women in chronic therapy, concentrations are higher than 300 pg/mg. In the athlete’s hair, no letrozole was detected along the 20 cm long hair shaft. Hair analysis turned to discriminate letrozole repetitive use vs occasional/inadvertent administration. The athlete received a reduced period of ineligibility.

For HCTZ, ingestion of contaminated supplements produced urinary concentrations higher than the WADA reporting limits up to 24 hrs; in the hair, collected after 15 days, no HCTZ was found. In the athlete’s 5 cm long hair no HCTZ was detected. The plausibility of the observed urinary concentrations with the supplement ingestion yielded a reduced period of ineligibility.

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As to case 5, the absence of all testosterone esters and exogenous steroids in the athlete’s hair supported a single exposure with null doping efficacy, but the athlete was deemed ineligible for the maximum period.

Discussion

The retrospective reconstruction of all the details of a non-voluntary exposure is extremely difficult. Different biological matrices must be analyzed, including food, beverages, and nutritional supplements, but since an AAF is notified several days after the collection of control samples some precious body of evidence may be lost. Excretion studies must often be envisaged; hair analysis is useful in ruling out a continuous (and efficient) doping use of prohibited substances.

Conclusions

When an athlete inadvertently ingests or is exposed to a prohibited substance, it is possible to demonstrate his/her reduced fault or negligence by applying toxicology methods but the success depends on the case, the circumstances, and a fair hearing process.
Abstract ID 536  
Towards non-targeted LC-HRMS screening for improved drug surveillance.  
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Aim  
The Australian Thoroughbred Racing Industry is largely a spectator sport with 4.5 million attendees annually and contributes $5 billion in value to the economy. New psychoactive substances are a major issue as they are constantly changing and threaten the integrity of the sport thus highlighting a major limitation on current screening methods. Synthetic opioids are a common source of doping due to the analgesic and stimulating effects seen in Thoroughbred horses. A new global approach to drug detection is a metabolomics workflow which aims to detect endogenous changes in molecular entities, such as metabolites and adducts, which relate to the function and metabolism of the drug within the body in question. The aim for this project was to utilise detection methods to monitor banned substances and their metabolites in order to create a metabolomics approach to identify biological passport changes due to doping.

Methods  
An administration study of butorphanol was conducted on twelve horses and spiked butorphanol plasma samples were used to complete the study. An analysis workflow consisted of sample preparation, liquid chromatography-quadrupole time of flight-mass spectrometry analysis and data processing. Sample preparation included protein precipitation, solid phase extraction, drying and reconstitution. The chromatographic analysis utilised an Agilent 1290 Infinity II coupled to an Agilent 6545 quadrupole time-of-flight mass detector. Data processing consisted of 3 steps; quantitative analysis, molecular feature extraction and statistical profiling.

Results  
The method was validated for butorphanol with a calibration range from 1 to 100 ng/mL (R²=0.9938). Limits of detection and quantification were calculated based on the standard deviation of the response and slope, and estimated to be 0.1 and 0.3 ng/mL, respectively. Accuracy was assessed through a residual plot which showed no evidence of bias through the random dispersion of data points. Precision was evaluated with 6 spiked samples ranging from 0.1 ng/mL to 100 ng/mL and was found to be within the acceptable range of 80-120%. Recovery and matrix effects were assessed to be 107.7% and 131.48%, respectively. Butorphanol was shown to be stable across a 4 week period study at 4°C and -20°C. Horse 2, 3 and 8 from the study were chosen for quantification. All three horses experienced a maximum concentration level, ranging from 74.7 ng/mL to 102.5 ng/mL, at the first sampling period of 0.08 hours. The plasma concentration levels gradually decrease over time which aligns with typical elimination curve studies conducted by Knych et al.

Discussion  
Butorphanol was no longer detectable through conventional methods after between 5 and 48 hours. The effects of doping were visualised through statistical modelling using Principal Component Analysis (PCA) on the endogenous metabolomics data. The PCA model was developed using the quantification data and required specific interpretations relating to the data for analysis. These interpretations included a categorical label specific to each horse, numerical grouping which was the sampling time point in hours and an activity grouping which related to whether the sample was negative, active or post. Negative samples were classed as samples taken prior to the administration of butorphanol. Active samples were classified as samples which still contained detectable amounts of butorphanol by conventional methods. Post samples were classified as samples taken after the butorphanol administration but amounts of the substance were no longer detectable. A statistical 2-way ANOVA analysis used the non-averaged interpretations to identify significant entities. The PCA model was created from the ANOVA test. The PCA aimed to detect all significant entities and further work will be conducted to identify these entities. The models showed a significant difference between samples which were collected from 12 to 96 hours post-administration and prior to administration.

Conclusions  
Doping continues to be a threat to the integrity of horse racing and thus new detection methods, such as metabolomics approaches with statistical profiling, can be implemented to combat this challenge. The greatest separation of the metabolomic changes between pre-administration and administration samples were seen when the butorphanol concentration was below the limit of detection established for conventional targeted testing methods. These findings allow for further work to broaden the modelling potential to other synthetic opioids and other drug classes for improved non-targeted screening methods.
Abstract ID 543
Microsampling as a promising anti-doping strategy for the analysis of peptide hormones and growth factors.

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Aim
The use of peptide compounds (hormones and growth factors) in sports is in constant evolution and several of them have been included in the World Anti-Doping Agency (WADA) prohibited list. Current sampling and analysis practices in the context of anti-doping activities are carried out on urine collected in- and out-of-competition, due to its convenient availability and relatively large sample volume. However, several of these compounds included in the WADA list of prohibited substances in sports could be susceptible to degradation, especially during sample shipping and conservation. It is thus evident that particular attention should be paid to sampling and storage procedures, in order to guarantee the highest stability of these substances.

The overall doping control system takes on great complexity from early sample collection steps in order to grant efficiency and reliability of the testing system. The availability of increasingly advanced instrumentations and the progressive automation of laboratory processes are not sufficient to ensure reliable analytical results, as they need to be supported by specific, suitable pre- and post-analytical phases. Sample collection, transfer and storage are indeed crucial steps for the success of anti-doping analyses and the needs generated by the high-quality standards and requirements pose many challenges for bioanalytical laboratories. The main purpose of this research was to establish and validate feasible but reliable protocols for the collection of urine microvolumes in dried form, that would be stably storable and shippable with no particular precautions.

Methods
The study involved the use of miniaturised dried urine samples, obtained for example by using 10-μL devices for volumetric absorptive microsampling (VAMS), by means of streamlined workflows developed ad hoc for the high-throughput LC-HRMS analysis of peptide compounds (hormones and growth factors), relevant to doping practice. VAMS is based on the accurate absorption of a fluid sample volume onto a porous substrate, by wicking. This microsampling strategy has already shown several significant advantages in terms of collection, sample treatment and also analysis. Sampling workflow is more feasible than currently available techniques, as the sampling device can be directly applied to the sample surface just for 2 seconds and does not require the collected material to be transferred into tubes or cards. Sub-sampling and additional purification steps can be avoided because the collected volume is the one to be analysed and its adsorption on VAMS tip represents a pretreatment starting step able to maintain matrix interfering components adsorbed onto the sorbent.

Results
A mid-term stability study was performed on the following twelve peptides: GHRP-1, GHRP-2, GHRP-6, hexarelin, alexamorelin, triptorelin, AOD9604, CJ-1293, desmopressin, TB-500, hCG and ACTH. It revealed that VAMS approach provided good results in terms of precision (RSD values always lower than 6.6%) and absolute recovery (always higher than 77%), with all the studied compounds recovered in the 80-90% range after 6 months. Moreover, dried matrix stability performance regularly outclassed the corresponding results from fluid samples, even when stored at -80°C. The matrix effect results were also satisfactory, being always in the 3.1-8.0% range. These data confirmed that such collection strategy could be a promising perspective in the research field of anti-doping analyses.

Discussion
The VAMS approach represents a promising sampling tool, allowing analyte stabilisation and leading to logistics advantages due to the small transported volume (10 μL of urine for each sample) and to the possibility to store the specimens at room temperature, with significant implications on the overall analysis feasibility, time and cost. Dried urine micromatrices proved to be attractive procedures when compared to fluid urine for the sampling, storage and testing of low-stability compounds during in- and out-of-competition controls. These data constitute a promising proof of concept for a potential use of these microsampling techniques in the context of anti-doping controls, in particular for those compounds more susceptible to degradation phenomena.

Conclusions
Bioanalysis is continually evolving and reaching new goals. Within this unending process, anti-doping analysis follows its own trajectory, but cannot go forward alone and has to use all of the bioanalytical strategies and tools, adapting them to sport testing. Among these, dried miniaturised matrices seem to have substantial advantages over wet ones, such as a generally better stability at room temperature and consequently more favourable behaviour during storage and transportation. For these reasons, dried microsampling and MS analysis represent probably the best prospect to become one of the golden standards at the forefront of anti-doping bioanalysis research. Coupling this setup with sample pretreatment and analysis automation and miniaturisation could produce important progresses in this field.

This study was carried out as part of a research project funded by the 2017 World Anti-Doping Agency (WADA) Research Grants [Research project reference number 17A20LM].
Abstract ID 30
Handling uncertainty of measurement in qualitative methods: a tool to help get ready for ISO 17025:2017 certification.
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Aim
Several qualitative decision point methods are used in forensic toxicology, comparing the unknown sample’s measurement against samples spiked at a cut-off, or threshold, concentration. Despite the binary nature of the results obtained (above or below cut-off), a comprehensive report should contain an evaluation of uncertainty. This view has been adopted by the International Organization for Standardization (ISO), which now requires an uncertainty evaluation for qualitative as well as quantitative methods in its most recent “General requirements for the competence of testing and calibration laboratories” (ISO 17025:2017).

It is known that qualitative decision point methods have an unreliability (UR) zone, akin to measurement uncertainty in quantitative methods. The UR zone describes a range of measurements (e.g., area, area ratio, height) which can be produced by samples at threshold concentration. Unknown samples producing a measurement below or above the UR zone can be confidently classified as below or above cut-off. The size of the UR zone should thus be evaluated to characterize uncertainty of measurement, but problematically, its size can change on a batch-to-batch basis, in particular with liquid chromatography systems coupled with tandem mass spectrometry. Our aim was therefore to develop advanced mathematical predictive tools to estimate accurately for each batch the size of its associated the unreliability zone.

Methods
To develop and validate an accurate model of the measurements’ behaviour, data was obtained using a qualitative decision point method targeting 40 drugs and metabolites. Cut-off and unknown whole blood samples were mixed with a solution containing 25 distinct stable isotope internal standards (IS) and extracted by protein precipitation. The diluted extract was then separated on an Agilent Zorbax Eclipse Plus C18 column using a 13 minute step/ramp gradient. The method was run on an Agilent HPLC 1200 or 1260 Infinity coupled to a Sciex 5500 QTRAP operated in positive scheduled multiple reaction monitoring (MRM).

Two data sets were constituted with different batches in which samples spiked at the cut-off concentration were analyzed k times. In the Study Data Set, \( i=13, k \) varied between 1 and 34 and \( k \) between 1 and 3; whereas in the Production Data Set, \( i=162, j=2 \) and \( k=1 \). The distribution and correlations of the obtained data (area ratios, areas) were examined to build a sound model of the measurements’ behaviour.

Results
Empirical probability densities derived from the data displayed multimodal distributions for peak area ratios and simple peak areas for both the analytes and ISs. It was observed that the areas were highly correlated between different batches, but the mean and variance differed. Thus, a shift in sensitivity (\( \delta \)) occurs between batches, displacing the distribution of measurements. This phenomenon is the root of the changing size of the unreliability zone (i.e., changing variance of the area ratio at the cut-off concentration).

Based on these observations, the following model was put forward: \( \Delta C \{ A_{ij} S_{1} - S_{c} \} = \delta \{ A_{ijk} S_{1} - S_{ijk} \} + \epsilon \), where \( A \) is the analyte area, \( S \) are the IS areas, \( C \) identifies data from the current batch and \( ij \) historical data. Stated this way, it becomes clear that the problem at hand is an imputation problem. \( \delta C \) can be estimated using the IS areas (current and historical). \( \delta C \) can then be calculated for all historical samples available highly correlated (\( \geq 0.9 \)) to the current batch data. By dividing those by the known area of the assigned IS, an area ratio set is constituted, from which the confidence interval is derived (\( \alpha/2 \) and 1-(\( \alpha/2 \)) percentiles).

Discussion
Similar imputation methods have been used in metabolomics and proteomics applications to correct for LC-MS/MS sensitivity shifts between batches. For small molecule applications, the difficulty lies in the presentation of measurements as area ratios rather than areas, which conceals the nature of the problem. But imputation can successfully be used to solve the problem at hand.

Leave-one-out cross-validation performed on the Study and Production Data Sets revealed excellent performance of the tool developed, with 94.5% and 93.9% of calculated confidence intervals containing the known current area ratio at threshold concentration (95% was the expected performance).

Using computer simulations based on experimental data, performance of the method was examined with a lower number of IS and historical samples, and less correlated data. The number of IS can be varied without reduction in method performance.

Conclusions
Using an imputation approach, the average area ratio at cut-off concentration and its confidence interval can be successfully predicted for the current batch. This confidence interval represents the unreliability zone at cut-off level of the method. Unknown samples can then be classified according to their measured analyte to internal standard area ratio as negative, likely negative, likely positive or positive. This procedure allows for an explicit evaluation of uncertainty and the appropriate limitations to be expressed on the results obtained, which is the fundamental goal of the ISO 17025:2017 requirements.
Abstract ID 65
Multidetermination of drugs of abuse in human hair using restricted access supramolecular solvent combined with LC-MS/MS.

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Aim
To develop a simple sample treatment method using volatile restricted-access supramolecular solvents for multi-residue micro-extraction of drugs of abuse in human hair and quantitation by liquid chromatography-tandem mass spectrometry.

To combine SUPRAS-RAM sample treatment to LC-MS/MS and validate the entire method in line with the requirements set forth by the European Commission decision 2002/657/EU.

To provide initial data base on simplification of hair sample treatment for forensic analysis.

Methods
Multidetermination of drugs of abuse in human hair plays a significant role in forensic science. Traditionally, drugs from hair have been extracted by techniques such as pressurized liquid extraction (PLE) and ultrasonic-assisted extraction (UAE) that require high volumes of organic solvents and are time-consuming. In addition, clean-up steps are required to obtain pure extracts which increases the consumption of organic solvents and costs and time of analysis. In this work, the combination of hexanol-based restricted access supramolecular solvent (SUPRAS-RAM) and liquid chromatography tandem mass spectrometry was used for the determination of 13 drugs of abuse from human hair. Efficient analyte extraction and sample cleanup was simultaneously achieved by vortex-shaking the hair sample with minute volumes of SUPRAS-RAM. The method was validated following the European Commission Decision 2002/657/EC.

Results
No signal suppression or enhancement was observed at the selected experimental conditions and quantitative recoveries (86-105 %) were obtained for all analytes. Method detection and quantitation limits were in the range (0.01-0.10 ng/g and 0.10-0.36 ng/g), respectively. The proposed method for sample treatment is simple and quick and meets the requirements of the European Commission decision to be used in real world.

Discussion
The supramolecular solvent selected for this study was synthesized from ternary mixtures of hexanol, THF and water. The formation of the SUPRAS occurred through well-defined spontaneous and sequential processes of self-assembly and coacervation (Figure 11A). First, a homogenous solution of hexanol in THF produced a colloidal solution of reverse micelles. Then environmental conditions were changed under addition of water, a solvent miscible with THF, to produce coacervation. Through this phenomenon, bigger aggregates were induced in the colloidal solution, which caused the spontaneous formation of oily droplets (i.e., coacervate droplets), that associated and produced conglomerates of individual droplets. The overall density of such conglomerates was different than that of the solution in which they formed, which facilitated their creaming and phase separation (SUPRAS).

Conclusions
The ability of supramolecular solvents to behave as restricted access materials for macromolecules allowed, simultaneously, the extraction of drugs and the removal of interferences from hair which simplifies sample treatment. The method here developed has valuable analytical and operational assets for the determination of drugs in human hair. It accomplished the European criteria regarding sensitivity (MDLs and MQLs range 0.01-0.10 ng/g and 0.10-0.36 ng/g, respectively), trueness (recoveries in the interval 86-105%) and precision (repeatability and reproducibility lower than 10 %). Furthermore, several samples can be simultaneously treated and it requires conventional lab equipment. So, the developed method is suitable for the determination of illegal drugs in human hair.
Abstract ID 129
Sensitive and selective screening of 143 fentanyl-related substances in biological samples using LC-Q-TOF-MS coupled with ion mobility separation.

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Aim
Overdose deaths by non-medical opioids, especially fentanyl-related substances, have reached epidemic levels. To circumvent drug controls, these substances are being illicitly synthesized by the introduction of slight modifications to functional groups of the fentanyl skeleton and it is difficult to differentiate these substances in forensic laboratories. Ion mobility is an additional dimension of separation, based on molecular size and shape. A value of collision cross-section (CCS) is an important distinguishing characteristic of an ion in the gas phase, being related to its chemical structure and three-dimensional conformation. The value of each ion can be determined by using its drift time through the ion mobility separation (IMS) device. Combining this IMS technique with an LC-Q-TOF-MS should provide analytical benefits, including the highly selective detection of various analogues in samples with a high matrix burden. In this study, for more sensitive and selective analyses, a screening method for 143 fentanyl-related substances in biological samples was developed using the LC-IMS-Q-TOF-MS system. Moreover, this method was applied to examine these substances in a human urine sample obtained from an emergency case in Japan, and the quantitative analysis of the substances detected in the sample was carried out.

Methods
The Q-TOF-MS and traveling wave IMS hybrid instrument (Synapt-G2-Si coupled to ACQUITY UPLC I-class system, Waters) was used for the screening analyses of the 143 fentanyl-related substances (including 24 metabolites and precursors). The substances targeted in this study were purchased from Cayman Chemical, USA. The Q-TOF-MS was performed in a "high definition mass E" (HDMSE) mode, which is based on sequential acquisition of mass spectra at low and high collision energies. This mode simultaneously provides accurate masses of the protonated molecule and fragment ions. In addition to retention times and accurate masses provided by the HDMSE, the CCS values were measured, and all data were stored in a mass spectra database for the fentanyl-related substances. Chromatographic separation was based on a Unison UK-phenyl column (3 μm, 2.0 mm x 100 mm, Intakt Co., Japan) using mobile phase of 0.1% formic acid and 0.1% formic acid/acetonitrile in a gradient mode. The test urine sample was obtained from the emergency case caused by the consumption of an illegal product in Japan. The urine samples were extracted with t-butyl methyl ether after enzymatic hydrolysis. For the quantitative analyses of the samples, an LC-MS/MS (XEVO TQ-S coupled to ACQUITY UPLC I-class system, Waters) in a multiple reaction monitoring (MRM) mode was used. Using this column, the chromatographic separation of the fentanyl-related substances was not enough and the retention times of the 143 substances ranged from 3.5 to 12.0 min even though the separation of various NPS (except the fentanyl-related substances) were almost satisfactory. Under the analytical condition using the phenyl column in this study, the separation of the fentanyl-related substances was greatly improved. Particularly, positional isomers which had the same protonated molecules and fragment ions (e.g. ortho-, meta- or para-fluorofentanyl) were well separated and it made possible more selective analyses of these substances.

Results
The screening method was established based on four index parameters; the accurate mass of the protonated molecule, the accurate masses of the fragment ions, the retention times (2.3-23.5 min) and the CCS values (142.1-193.3 Å²) of the 143 substances. Under the analytical condition used in this study, most substances were able to be identified with some exceptions. The detection limits of the control substances added to the control urine samples ranged from 0.5 to 10 ng/mL, and the recoveries of the substances were more than 60 %. The precision and accuracy of the data are almost satisfactory and are less than 20 % at the concentration of 10 ng/mL. Using the analysis in combination with the IMS, the background interferences on the mass chromatograms were reduced and the detection sensitivities of each substance were increased. These results led to the analyses narrowing down the candidate substances by library searching the mass spectral database for fentanyl-related substances. As a result of the screening analysis for the test urine sample, fentanyl, norfentanyl and beta-hydroxyfentanyl were detected. According to the quantitative analysis of the sample, their concentrations ranged from 4.3 to 22.3 ng/mL. Additionally, using the screening method for 500 new psychoactive substances (NPS) which was developed previously [R. Kikura-Hanajiri et al., the 56th TIAFT meeting], 4-fluoro-α-PHPP and 7 psychotropic drugs (including therapeutic drugs) were also detected.

Discussion
In the screening method for NPS developed previously, a CORETECS C18 column (2.7 μm, 2.1 mm x 150 mm, Waters) was used. Using this column, the chromatographic separation of the fentanyl-related substances was not enough and the retention times of the 143 substances ranged from 3.5 to 12.0 min even though the separation of various NPS (except the fentanyl-related substances) were almost satisfactory. Under the analytical condition using the phenyl column in this study, the separation of the fentanyl-related substances was greatly improved. Particularly, positional isomers which had the same protonated molecules and fragment ions were well separated and it made possible more selective analyses of these substances.

Conclusions
Acquiring the LC-Q-TOF-MS analytical data, combined with the added specificity from the ion mobility, made it possible to discount chromatographically co-eluting compounds and background interferences. The LC-IMS-Q-TOF-MS system in this study is useful for screening analyses of various analogues of fentanyl-related substances with low concentrations in biological materials.
Abstract ID 201
A Point System for “Identifications” in Forensic Toxicology Laboratories.

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Aim
This talk will discuss the development of a point-based system for forensic toxicology and demonstrate how it may be used in a laboratory setting.

Methods
Other fields of analytical chemistry have attempted to make the identification process more objective through a point-based system. In the United States, the Toxicology Subcommittee of the Organization of Scientific Area Committees (OSAC) drafted a document to do the same. The document sets minimum criteria, based on a point system, for analyte identification during forensic toxicology testing.

Results
Different analytical techniques were assigned points based on their general specificity. For example, colorometric tests (e.g., Fujiwara, TLC visualization techniques, Trinders) and non-instrument immunoassays (dipstick, urine cups) were awarded 0.5 points while instrumental immunoassays (e.g., ELISA, EMIT, CEDIA) were assigned 1 point. Likewise, chromatographic or electrophoretic separations were allotted 1 point and additional points are awarded for use of non-selective (+0.5), selective (+1), or mass spectrometric detectors. The points assigned to the use of mass spectrometers depend on whether they are low- or high-resolution, as well as the mode of operation (e.g., full scan, SIM, MS/MS).

Using the above point scheme, a sum of a minimum of four points are required to achieve identification of an analyte. These points must be achieved by combining no more than three different techniques. If mass spectrometry is not utilized, at least two different chromatographic separations shall be performed. Repetition of the same method does not earn additional points toward the total required for identification. Additional caveats include a requirement that all analytical methods used to generate identification points shall be properly validated; at least one chromatographic or electrophoretic separation technique shall be performed; and no more than two points shall be awarded for non-chromatographic mass spectrometry techniques.

Discussion
Traditionally, forensic toxicologists “screen” biological specimens for the presence of individual drugs, drug classes, or their metabolites to rule out the presence of analytes that are detected by these techniques or to indicate when further testing is warranted. The presence of analytes that “screen positive” is confirmed by a second analytical technique that is based upon one or more different chemical principles than the screening technique. It has been accepted that following this approach, adequate proof exists to declare the analyte as “identified” in the biological specimen. However, there are a wide array of techniques and instrumentation in forensic toxicology laboratories and each technique offers a range of identification potential. Assigning points to analytical techniques allows a forensic toxicology laboratory use a combination of validated analytical techniques to more objectively evaluate their analytical schemes used to identify drugs, metabolites, and poisons.

Conclusions
The proposed point system allows laboratories to evaluate each analytical technique to determine if their testing regimen is sufficient to meet or exceed the minimum four-point threshold for identification.
Abstract ID 222
Searching matches for time-of-flight spectra in an Orbitrap spectral database: results from a study simulating tentative identification of unknowns.

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Aim
Identification of true unknowns remains a major challenge within small molecule analysis. In forensics, the still strongly broadening selection of new psychoactive substances (NPS) sets continuous need for unknown identification. However, increasing demands rise in various additional fields as well, including metabolomics, doping, and food safety, emphasizing the extent of the challenge. The limited availability and often delayed delivery of certified reference material forces laboratories to look for alternative solutions. Tentative or putative identification without reference standard is not accepted as a standard procedure within the ISO standard, and for true identification a reference standard is still always required. However, alternative approaches and tentative identification can be useful and even crucial in certain occasions, for example if an epidemic of a potentially lethal NPS analog is suspected. In this study, tentative identification of unknowns was simulated by searching time-of-flight (TOF) spectra of known substances against an open access Orbitrap-based spectral database “mzCloud”.

Methods
As part of routine laboratory database updates, 38 new NPS including synthetic cannabinoids, cathinones, fentanyl derivatives and designer benzodiazepines were characterized in detail. Reference material was purchased from Cayman Chemical (Ann Arbor, MI, USA), Cerilliant (Round Rock, TX, USA), Lipomed (Arlesheim, Switzerland), and Chiron (Trondheim, Norway). In addition, seizures certified by the Finnish Customs were used. Characterization included, among other things, acquisition of MS and bbCID (MS/MS) spectra data sets at mass range of 50-750 Da for all molecules. The instrument was a Maxis Impact (Bruker Daltonics, Bremen, Germany) QTOF combined with an Ultimate 3000 ( Dionex, Thermo, Sunnyvale, CA, USA) UHPLC. The bbCID-spectra of the molecules were converted to ASCII xy-files for the “mzCloud”-search. “mzCloud” is an open access spectral database available at https://www.mzcloud.org/. It includes a full MSn characterization of more than 17000 compounds acquired with an Orbitrap instrument.

In order to simulate the case of a true unknown, the xy-converted bbCID spectra acquired with the TOF were imported to “mzCloud” and searched against the database in Search Spectrum-function with the HighChem HighRes- and Opt. Dot Product-algorithms. The m/z search window was set to 10 mDa, and the precursor candidate accurate mass (from the TOF MS data set) was inserted as well.

Results
Of the 38 compounds studied, 25 were identified and reported correctly as the first candidate, 3 as the second candidate and 2 as the third candidate. For 8 compounds the database did not contain reference spectra, including both “established” (available at least since 2016, 4/8), and “recent” (only a few hits in a google search 4/8) compounds. As a result, 83% of first candidate search results were correct. This is an unexpectedly good result, as the spectra were acquired with two different techniques (TOF and Orbitrap), and the TOF spectra were acquired in bbCID-mode, which produces mixed spectra, instead of true selective MS/MS spectra. The second and third position matches were all received for compounds with positional isomers included in the database. The spectral match factor varied from 90.3% to 63.4% for the HighChem HighRes-algorithm and from 98.6% to 62% for the Opt. Dot Product algorithm for the correct identifications. The HighChem HighRes algorithm produced correct identification more often, but with lower average match. The “mzCloud” search result view included visualization of the measured spectrum, the reference spectrum and their difference. Thus, in addition to the numerical match value, the visual inspection of the spectral difference served as a conclusive tool. The following criteria were set for positive identification: spectral match > 80%, no other matches within 2%, positional isomers cannot be differentiated. The tentative identification should be confirmed with a reference standard as the final step of the identification procedure.

Discussion

Conclusions
The simulation of true unknown search proved that the presented approach is applicable as an additional tool in the process of true unknown tentative identification. Despite the fact that the experimental data and the database data were acquired with different techniques, the search result was reliable, excluding positional isomers. However, for 21% of the compounds studied, no reference spectra existed in the database, and the lack of reference spectra still remains the main obstacle in tentative and putative identification. Open access databases are a valuable add-on to the field.
Abstract ID 303
Analyte quantification in Dried Blood Spots deposited on non-standardized materials with subsequent blood volume estimation.

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Aim
Development and validation of a quantitative method for the analysis of codeine, morphine, dihydrocodeine (DHC), benzoylecgonine (BE), ecgoninemethylester (EME) and methadone in dried blood spots (DBS). Determination of DBS blood volumes and considerations whether conclusions can be drawn on drug concentrations in blood traces found on different materials at a crime scene.

Methods
Blood samples were spiked with a standard solution mixture containing the analytes of interest and prepared as DBSs on six different materials. Materials, chosen due to their common appearance at crime scenes, included printer paper, wood, tile, carpet, and fabrics made of cotton, polyelastane (PE), or a cotton-polyelastane composite. DBSs dried in an ambient environment (48 h) and were reconstituted in toto in Tris-buffered-saline/Tween 20 (0.1 % v/v) solution. 500 µL of the resulting DBS-solution were spiked with internal standard mixture, followed by SPE and derivatization. These solutions were then used for and quantification of the analytes of interest via GC-MS/MS. 20 µL of the DBS-solution were prepared with Drabkin reagent for photometric hemoglobin (Hb) determination via the cyanhemoglobin (HiCN) method. Validation was performed according to the GTFCCh guidelines: Accuracy was determined by intra-day and inter-day precision (RSDr and RSD(T)) as well as trueness (bias), following the criteria of ≤ ± 15 % (≤ ± 20 % at LOQ). Extraction efficiency was examined at two concentration levels. The presented method is intended to extend the applicability of dried blood spot analysis for use in crime scene investigation.

Results
Within the foregoing validation of analyses of DBSs prepared on printer paper, sufficient validation results for linearity, accuracy, selectivity and extraction efficiency were achieved for all mentioned substances. The RSDr values were < 10 %, RSD(T) < 15 % for all analytes in this validated method. Biases of < ± 15 % could be observed for codeine, morphine, DHC and BE. Biases of -13.9% to -29.3% occurred for methadone and EME. Preliminary results with DBSs on other materials revealed variations between the materials and analytes with respect to accuracy data. Codeine and DHC show satisfactory results for accuracy data with RSDr ≤ 10 % and RSD(T) < 15 % and biases of < ± 15 % for all materials. RSDr and RSD(T) of < 15% could also be achieved for all other analytes and materials, with differing biases. Thus far, biases of -12% to -20% could be estimated for EME on cotton, PE, and cotton-PE composite, and -21% to -29% for the remaining materials. Morphine and methadone both showed satisfactory results for DBS prepared on tiles (biases < ± 15 %), but varying biases of approximately -24% to -40% for EME and -16% to -27% for morphine dependent on the other materials. Benzoylecgonine could be found to lead to biases within the criterion for all materials except for carpet and wood. For the latter, biases between -17% and -20% occurred, slightly exceeding the criterion. Extraction efficiency has still to be tested. A correlation between the measured Hb values and the initial spot volumes was ascertained (R2= 0.9995) and RSDr/RSD(T) of < 10 % and biases of < ± 10 % were shown for carpet, wood and tile, whereby the materials cotton, PE, and cotton-PE composite showed biases < ± 15 % Small differences in hemoglobin measurements, dependent on the carrier material, could be observed, but acceptance criteria were still fulfilled for all materials.

Discussion
The validation data for DBS prepared on printer paper lead to satisfying results regarding the predefined criteria and thus to a quantitative method for the determination of the analytes in question. For other materials, preliminary results have to be considered critically, dependent on analyte and carrier materials. The determinability of analytes, such as methadone and morphine, appear to be more susceptible to varying carrier materials than EME and BE, whereas codeine and DHC seem to not be susceptible to such changes. The extractability of blood from the different carriers might be an explanation for the slight differences in hemoglobin measurements, but also for the observed differences in the analyte concentrations. Analyte stabilizing effects on the dried matrix might also be constrained by the differences in blood distribution and the differences in absorption of blood on the materials, and the resulting drying speed.

Conclusions
A method for the determination of methadone, opiates and cocaine metabolites in DBS was developed and validated for DBSs prepared on printer paper and is still to be validated for different non-standardized carrier materials. Back-calculation of the initial blood volumes of whole DBSs was possible on print paper, demonstrating its suitability for estimating the drug concentration in the spot. Moreover, this method seems to be mostly independent of the carrier material. To date, blood samples in physiological hemoglobin ranges enable a close estimation of the drug concentration in the spot and thereby, in the blood before drying.
Abstract ID 324

ToF-SIMS and MALDI Analysis of Longitudinal- and Cross Sections of Single Hairs as a Tool for Gaining Insight into the Contamination Issue.

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Aim
Sample preparation in liquid chromatography-mass spectrometry (LC-MS) hair analytics typically includes wash protocols to remove contaminations from sebum/sweat or external sources. Recently, wash protocols had been suspected of washing analyte contaminations into the inner hair matrix, thus questioning the validity of hair testing. In the present study, the use of time of flight – secondary ion mass spectrometry (ToF-SIMS) and matrix assisted laser desorption ionization-mass spectrometry (MALDI-MS) in combination with longitudinal- and cross sectioning of hairs and (extreme) decontamination should allow new insights in that issue.

Methods
Zolpidem, cocaine- and methadone-soaked hair samples (12 mg hair in 2 mL water with 1 µg/ml analyte for 1 h), samples from respective consumers and cocaine powder contaminated single hairs were investigated without and after decontamination with an in-house protocol (Briefly, 10 times for 2 minutes in 5 mL methanol, followed by an extended wash of 18 hours in 5 mL methanol). Wash solutions were also analyzed using LC-MS/MS.

Single hairs were longitudinally sectioned with a blade on a custom-made aluminum block. Grooves with different depths should allow to section a hair at approximately half of its thickness. For cross sections, single hairs were embedded in a mixture of 2% (w/v) carboxymethylcellulose (CMC) in water followed by instant freezing with liquid nitrogen. Sections were cut using a cryostat (LEICA CM3050 S). Hairs were coated with the MALDI matrix alpha-cyano-4-hydroxycinnamic acid (CHCA). MALDI-MS signal intensities were acquired on a Sciex Flashquant Workstation® (Sciex, Darmstadt, Germany) and on an iMScope TRIO (Shimadzu, Duisburg, Germany).

For images with higher spatial resolution, ToF-SIMS was used in spectrum or imaging mode (TOF.SIMS 5; IONTOF GmbH, Muenster, Germany).

Results
Longitudinal sectioning of hairs dramatically increased sensitivity, enabling the detection of even single-dose administrations of zolpidem using MALDI-MS. Cross sections of single hairs offered the possibility to establish contamination profiles using ToF-SIMS and MALDI-MS. Insights into the incorporation of xenobiotics into hair were possible along with the study of contaminant distribution in relation to decontamination processes. Zolpidem soaked hairs could be cleared almost completely with the extreme in-house wash protocol. In contrast, zolpidem from consumer hairs, supposedly strongly bound to inner hair structures, could not be completely removed. As visualized with MALDI-MS, methadone and cocaine consumer hairs markedly differed from zolpidem consumer hairs in their degree of contamination via sweat and sebum. Extreme external contamination, e.g. by actively rubbing cocaine powder into head hair, lead to hair samples that resisted complete decontamination. In these cases, MALDI-MS results were very similar to those from actual consumer hairs.

Discussion
The combination of longitudinal- and cross sections of single hairs analyzed by MALDI-MS and ToF-SIMS provided new insights into contamination/decontamination processes. The resulting incorporation of a xenobiotic from external sources into hair seems to differ from an analyte having been incorporated from the bloodstream. Thus, the usage of decontamination protocol in routine hair analysis is useful. Under certain conditions (e.g. after use of powdered drugs), even the strongest decontamination protocols (already leading to partial extraction) failed to differentiate between consumption and contamination. Therefore, criteria such as cut-off values and metabolite ratios remain indispensable in hair analysis, to increase the level of confidence.

Conclusions
The applied techniques could help to differentiate between consumption and contamination. Extreme external contamination (e.g. from powdered drugs) can exhaust the capacity of the strongest decontamination protocol, thus preventing differentiation. These findings emphasize the importance of further analytical criteria such as metabolite ratios to gain increased confidence in the interpretation of the analytical results.
**Abstract ID 357**

*What we thought we knew – Master class on MSMS-spectra interpretation of fentanyl metabolites.*

**Svante Vikingsson**

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**Aim**

In general, very little is known about the toxicokinetics of new psychoactive substances and fentanyl analogs are no exceptions. Much of what is known comes from metabolism studies, analyzing either incubates from in vitro model systems or authentic urine samples by high-resolution LC-MSMS. These studies typically do not have access to reference materials and the identification of the metabolites is based solely on the interpretation of the data and in particular the fragments of the product ion spectra (MSMS-spectra).

Over the years, a number of rules-of-thumb have developed to aid in the interpretation process. For example, a fragment identical to that found in the MS-spectra of the parent indicate that part of the molecule is unmodified or that a loss of m/z -18 indicate the loss of water. Such a loss from a hydroxylated metabolite indicate that it is aliphatic as such a loss cannot happen from an aromatic ring.

In this project, β-hydroxy and 4-hydroxy metabolites of fentanyl and five different fentanyl analogs were synthesized providing us with the opportunity of analyzing MSMS-spectra from metabolites with known structures. The aim was investigate if fragmentation of β-hydroxy and 4-hydroxy metabolites (both being major metabolites of many fentanyl analogs) follow expected patterns and can be accurately predicted using the rules-of-thumb.

**Methods**

β-hydroxy metabolites are major metabolites of many fentanyl analogs and are hydroxylated on the ethyl linker connecting one of the phenyl groups to the piperidine ring. 4-hydroxy metabolites are also major metabolites hydroxylated on the para-position of the phenethyl moiety. β-hydroxy and 4-hydroxy metabolites of fentanyl as well as acetyl-, acryl-, cyclopropyl-, isobutyryl- and 4F-isobutyrylfentanyl were synthesized in-house.

The metabolites were diluted in mobile phase and analyzed using LC-QTOF-MS on an Agilent 6450 QTOF. The separation was conducted using different gradients of 0.05% formic acid in 10 mM ammonium formate and 0.05% formic acid in acetonitrile on a Waters Acquity HSS T3 column (150 × 2.1 mm, 1.8 µm). MS-spectra were collected using data-dependent auto-MSMS.

**Results**

The MS-spectra of six β-hydroxy metabolites and the spectra of six 4-OH metabolites from fentanyl and different fentanyl analogs were analyzed. Major fragments of the β-hydroxy metabolites included m/z 204 (phenethyl-piperidine), the corresponding water loss m/z 186 and the tropylium cation, m/z 91, formed from the phenethyl moiety. Fragments m/z 132 and m/z 174 do not correlate to distinct parts of the molecule and should be interpreted with caution. The spectra also contained fragment m/z 105, which is consistent with an unmodified phenethyl moiety, at greater intensity than the expected fragment m/z 121, consistent with a hydroxylated phenethyl moiety.

Similarly, the MSMS spectra of the 4-OH metabolites were dominated by m/z 121 (hydroxylated phenethyl) together with m/z 204 and 84 (piperidine ring). In addition, a minor fragment ion at m/z 103, indicative of a water loss from m/z 121.065, was observed. According to the rule-of-thumb a water loss peak is expected from aliphatic hydroxylations but not from aromatic, which is inconsistent with the actual structure of the 4-hydroxy metabolites.

**Discussion**

Both fragment 105 the water loss from 4-hydroxy metabolites are unexpected given the structure and it is not clear how they are formed. Using our rules-of-thumb on these fragments would lead us astray and potentially lead to misinterpretations. Given that both β-hydroxy and 4-hydroxy are major metabolites of fentanyl analogs this could affect our understanding of these compounds.

These examples illustrate the limitations of metabolism investigations using LC-QTOF-MS and we must all remember that without proper identification using reference materials, proposed structures must be interpreted with care and in light of the limitations of the method. In our opinion, limited weight should be placed on minor fragments and fragments where the exact structure or mechanism is not well understood (such as m/z 132 and 174).

**Conclusions**

By synthesizing and analyzing β-hydroxy and 4-hydroxy metabolites of fentanyl and five fentanyl analogs, it was shown that both these type of metabolites produce MSMS-spectra containing unexpected fragments, potentially leading to the risk of misidentifying metabolites.

β-hydroxy metabolites are modified on the phenethyl moiety but produce fragment m/z 105, indicating that moiety to be unmodified. Similarly, 4-hydroxy is an aromatic hydroxylation but still show water loss consistent with aliphatic hydroxylations. The mechanisms are not known. The examples illustrate that metabolite identifications based on MS-spectra should be regarded as suggested structures and interpreted with caution, especially for minor fragments.
Abstract ID 391
A Novel Serotonin Receptor Bio-Assay For The Activity-Profiling And Detection Of Hallucinogenic NPS?
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Aim
Designer hallucinogens comprise the third largest portion of reported Novel Psychoactive Substances (NPS), only preceded by cathinones and synthetic cannabinoid receptor agonists. Despite broad structural differences, they share activation of serotonin (5-HT2AR) receptors (5-HT2ARs), which belong to the G protein-coupled receptor (GPCR) class, as their main pharmacological target.

Bio-assays employing the cannabinoid receptors and the mu-opioid receptor, recently developed by our lab, have been shown to be useful in activity-profiling of NPS, but also in the screening of biological matrices in search of those substances. This activity- rather than structure-based approach, allows for the detection of new, undescribed compounds. Since hallucinogenic NPS emerge at a rapid pace, characterization and activity-based detection were of interest in this context.

Methods
Assay methods: The newly developed bio-assay utilizes transiently transfected HEK293T cells in 96-well plates, in which activation of the 5-HT2AR, fused to one part of a split nanoluciferase, leads to recruitment of β-arrestin2, fused to another part. Application of 5-HT2AR agonists results in receptor activation, leading to functional complementation of the nanoluciferase, which can easily be monitored via luminescence.

Sample preparation: Plasma samples were extracted via liquid-liquid extraction, using 95:5 ethylacetate/isopropanol as the extraction solvent. The supernatant was evaporated to dryness under nitrogen, and reconstituted in HBSS. In case of MAO-A (monoamine oxidase-A) treatment, 5 µL (5 U) of the enzyme solution was added, followed by incubation for 1 h at 37 °C. For LC-HRMS, methanol was used for additional dilution and protein precipitation.

LC-HRMS: A Phenomenex Kinetex 2.6-µm C18-column (3 x 50 mm) was used in an Agilent 1290 Infinity LC system, coupled to a 5600 QTOF HRMS from ABSciex, with an electrospray ionization (ESI) source, and Analyst TF 1.7.1 software.

Results
The optimal combination of receptor and β-arrestin2 fusion-proteins was determined, using LSD as a reference hallucinogenic compound. The optimized bio-assay demonstrated a concentration-dependent response for LSD, and was therefore used to assess receptor activation of three 2C compounds (2C-B, 2C-C and 2C-I) and their corresponding NBOMe-derivatives. This resulted in EC50 values between 3.16nM and 8.46nM for the 2C group. The NBOMes were more potent, with EC50 values between 0.758nM and 0.819nM – LSD was taken along as a reference, having an EC50 of 6.41nM. Besides the higher potencies, also the efficacies of the NBOMes were higher than those of their 2C counterparts.

In a next step, we attempted to apply this bio-assay for the screening of biological samples. However, blank plasma already showed receptor activation. Via an antagonist experiment, we confirmed the specificity of this activation, meaning that there was an 5-HT2AR agonist present in plasma. Suspecting that the presence of endogenous serotonin was accountable for the activation profiles, regular plasma extracts were incubated with MAO-A (an enzyme specific for serotonin degradation). A 1-hour incubation with MAO-A indeed was capable to virtually completely annihilate the receptor activation. LC-HRMS analysis confirmed the presence of serotonin in blank plasma and a strong reduction after treatment with MAO-A. Furthermore, while MAO-A treatment could not abolish 5-HT2AR activation by LSD, it was effective at abolishing activation by 2C-B. This demonstrates that MAO-A treatment of plasma is not an option when aiming at setting up a universal screening assay for 5-HT2AR activity in this biofluid.

Discussion
The relevance of 2C hallucinogens and their NBOMe derivatives was emphasized by Risk Assessment Reports of the EMCDDA. In the new assay reported here, this risk was confirmed, as the potencies and efficacies of the tested compounds were in the same range as LSD for the 2C group, and even higher for the corresponding NBOMes, confirming the higher potency of the latter found in in vivo animal studies. Depending on the assay platform used, divergent potency and efficacy ranking orders were obtained for the different compound categories, making it rather complex to compare our data to the available literature.

Due to the presence of the endogenous compound serotonin, the application of the bio-assay as a screening tool for biological matrices is not as straightforward as the existing cannabinoid and opioid reporter assays. The routine implementation of a MAO-A incubation step to overcome the issue of endogenous serotonin would severely complicate the interpretation of results, due to the degradability of 2C (and possibly other) hallucinogens and the presence of MAO-A inhibitors in medicine (e.g. antidepressants) and in hallucinogenic decoctions (e.g. ayahuasca).

Conclusions
We successfully developed a new 5-HT2AR reporter assay. The applicability of this system will primarily lie in its use for the activity profiling of newly emerging hallucinogenic NPS, a scarcely described group of compounds. This approach was already shown to be successful with other GPCRs. In combination with other assays, this bio-assay may allow better insight into the signaling elicited by different hallucinogens. The implementation in an activity-based screening context is impeded by the presence of endogenous serotonin.
Abstract ID 396
P4 strategic production of NPS reference materials – Eurostars PSYCHOMICS Project.

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Aim
New Psychoactive substances (NPS) have been emerging onto the recreational drug scene at an unprecedented rate. To keep up with the pace, new reference methods and reference materials are required to enable accurate detection of drug use by law enforcement authorities and forensic toxicology institutions, not only to provide criminal evidence but also to link these unregulated substances to severe health events and deaths.

Despite concerted efforts by reference material manufactures, the rate at which new NPS emerge significantly outpaces the development of counterpart Reference materials. The current paradigm for reference material production is based on a reactive process, where laboratories and authorities are always one step behind the illegal producers. Through the Eurostars PSYCHOMICS project, Chiron AS and collaborative partners aimed to develop a disruptive synthesis process for producing certificated reference materials (CRM) for NPS, their metabolites, and labelled internal standards, promoting a significant paradigm change in forensic toxicology.

Methods
The approach adopted in the PSYCHOMICS Project is a Predictive Parallel Production Platform, P4 strategy, which allows a significant increase in the number of available CRMs for NPS compounds. The P4 approach relies on an efficient, simultaneous and larger scale synthesis of several compounds, targeting not only already reported NPS, but also those which are likely to appear in the market based on chemical similarities to known compounds or to known structure-activity relationships.

Another pipeline for P4 approach focuses on improved understanding of NPS metabolism. This arm of the project, undertaken in Collaboration with the Linköping University and the Swedish Board of Forensic Medicine, RMV, utilizes a combination of hepatocytes and microsome-based process to predict metabolism pathways. Targeting metabolites enables better detection of extensively metabolized compounds, that might otherwise go undetected, prolongs the detection windows and provides more robust confirmation in the presence of parent compounds.

Both deuterated and 13C-labelled internal standards (ISs) were synthesized, and the relative advantages were studied on chromatographic analysis.

Results
To date, the PSYCHOMICS P4 strategy has resulted in the development of between 100-130 NPS reference materials each year during the project period. The CRMs developed in the project include synthetic cannabinoids, cathinones, fentanyl, amphetamines, NBOMs, tryptamines and designer benzodiazepines. They encompass the native drugs, their metabolites and deuterated and 13Clabelled ISs.

Different families and series of NPS compounds share similar precursors, being organized in tree-like synthesis Cascades. One of examples is the synthesis of Cumyl-PeGACLONE and analogues. Cumyl-PeGACLONE (2-Cumyl-5-pentyl-gamma-carbolin-1-one) was identified in 2016 through systematic online monitoring performed as part of an EU-financed Profiling Project. The compound features a novel tricyclic gamma-carbolinone (1-oxo-gamma-carboline) core structure and appears to have been specifically designed to circumvent generic bans on SPICE compounds. It is reasonable to anticipate the emergence of analogues. Modification of the structure through substitution on the two nitrogens led to the synthesis of 5F-Cumyl-PeGACLONE. We also postulated three other potential analogues and proposed the nomenclature as Benzyl PeGACLONE, Cumyl CHMGACLONE and MMB-FUBGACLONE. Reports of 5F-Cumyl-PeGACLONE are already emerging, and Cumyl CHMGACLONE was also reported later as Cumyl-CH-MeGACLONE.

Another example is the synthesis of Carfentanil and the prediction of possible analogues. Simple, scalable, late-stage diversification routes are available to synthesize new designer fentanyl, carfentanils, major metabolites and stable isotope labelled ISs. A range of Carfentanil derivatives (isobutylryl- 2-floro-Carfentanil for example) can be produced in the same procedure as for Carfentanil. Metabolism study on designer fentanyl and synthetic cannabinoids reveal the most abundant metabolites for targetted synthesis; isotope labelled ISs were also synthesized by labelling on the most detected fragment moieties. Urinary metabolites of fentanyl and synthetic cannabinoids were identified, and the major metabolites were synthesized in relatively large scale.

From the comparation study on deuterium and 13C-labelled ISs, chromatographic resolution between deuterated benzodiazepines and native (unlabelled) benzodiazepines increased with the number of 2H-substituees, whereas 13C6-labelled benzodiazepines and native benzodiazepines were found to co-elute. MS/MS analyses of benzodiazepines and their stable isotope variants showed that 13C-labelled ISs gave better results With regarding ion suppression effects than 2H-labelled ones. The findings, support previous studies on amphetamines, shwing that 13Clabelled ISs are preferable for analytical purposes.

Discussion
The P4 Strategic production enabled the efficient synthesis of large number reference materials of both existing and predicted NPS and associated metabolites. After the success of PSYCHOMICS project, the P4 approach will be continued, a new Eurostars Application (NPS-REFORM) has been submitted to develop a scale-up production platform of NPS Reference materials and proactive Methods for prediction and monitoring of the flow of potent NPS.

Conclusions
Detection of Fentanyl and Methamphetamine using surface enhanced Raman spectroscopy.

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Aim
Raman spectroscopy is used as a non-destructive technique for the analysis of unknown solids, presumed to be illicit drugs, by agencies such as homeland security and police affiliated laboratories. Although the application to identifying unknown solids is useful, more sensitive Raman methods, such as surface enhanced Raman spectroscopy (SERS), could allow for this technique to be expanded to the detection of lower levels of analytes in forensically relevant matrices. The aim of this study was to develop a method using SERS for the detection of low levels of fentanyl and methamphetamine, in oral fluid.

Methods
Raman analysis was completed with the benchtop Renishaw inVia Raman microscope with 785 nm near infrared laser. The instrument conditions included the range of 550-2000 cm⁻¹, microscope magnification 20x, laser power 100 mW and pinhole in. Commercial silver SERS substrates were used for enhancement and the surface structure determined using scanning electron microscopy (SEM). The concentrations tested ranged from 5 ppm to 1 ppb for both fentanyl and methamphetamine. Spiked and blank oral fluid (100 µL) was extracted and centrifuged for 2 minutes at 2000 rpm, 5 µL aliquot of the extract was deposited onto the substrate.

Results
Preliminary results have shown that methamphetamine was easier to detect than fentanyl. Methamphetamine was able to be detected down to 1 ppb consistently in the standard solutions and 10 ppb in oral fluid. At a concentration of 1 ppb, in the standard solutions, five peaks could be attributed to the methamphetamine. Fentanyl was able to be detected at 1 ppb in standard solutions by the peaks at ~744 cm⁻¹, ~856 cm⁻¹ and ~1001 cm⁻¹. In the oral fluid, fentanyl was able to be detected down to 10 ppb. The surface structure of the substrate was found to be silicon pillars covered in silver. These pillars leaned together upon solvent evaporation, trapping the analytes in the area of enhancement.

Discussion
The silver substrate was more suitable for the analysis of the methamphetamine analyte in both standard solutions and oral fluid. These results have shown promise for the use of SERS for the low level detection of methamphetamine and fentanyl in a forensically relevant matrix. The oral fluid extracts had lower signal intensities due to the lower amounts of clustering of the pillars as the organic solvents dry quicker than the aqueous standard solutions. Implementation of this work into routine analysis would require testing with authentic oral fluid samples, assessing the compatibility of other drug analytes and using handheld/portable Raman instruments.

Conclusions
Surface enhanced Raman spectroscopy has been shown to be effective for the detection of methamphetamine and fentanyl. Future work should include expansion to using handheld and portable spectrometers before possible implementation into routine testing.
Abstract ID 440

Enantiospecific synthesis, separation, detection and pharmacological assessment of carboxamide-type synthetic cannabinoid receptor agonists in seized samples.

Lysbeth Antonides

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Aim

Synthetic cannabinoid receptor agonists (SCRAs) are the largest group of illicit psychoactive substances reported to early warning systems and many of the most potent and prevalent compounds are chiral. The aim of this study was to synthesise a series of enantiopure carboxamide-type SCRA reference materials, develop analytical methods to differentiate SCRA enantiomer pairs and to determine their relative prevalence and variability in seized samples. The influence of chirality on the intrinsic metabolic stability of chiral SCRs was assessed and the effect of chirality on potency and efficacy at the cannabinoid G-protein coupled receptors (GCPRs CB1 and CB2) was studied to further elucidate structure activity relationships (SARs). Methodology combining chemical analysis and activity-based bioassay data to simplify the communication of relative toxicological and pharmacological harm of seized samples is proposed.

Methods

Carboxamide-based SCRAs including AMB-FUBINACA, AB-FUBINACA, SF-MDMB-PINACA (SF-ADB) and AB-CHMINACA, were synthesised and characterised. A quantitative gas chromatography-mass spectrometry (GC-MS) method for the achiral analysis of SCRs in herbal materials was validated and applied to seized samples. Six chiral HPLC stationary phases were assessed and a chiral high-performance liquid chromatography separation with photodiode array and quadrupole time of flight mass spectrometric detection (HPLC-PDA-QToF-MS) method was developed, validated, and street-and prison-seized samples analysed. Live cell-based reporter assays monitoring protein-protein interactions via NanoLuc Binary Technology were used to assess the biological activity of the synthesised reference compounds. Receptor activation was evaluated via the interaction between βarr2 and CB1 and CB2 and EC50 (a measure of potency) and Emax values (a measure of efficacy) determined. Data from achiral, chiral and bioassay analyses were combined to calculate an estimated intrinsic potency value for each sample analysed as a method to communicate the relative harms of different samples.

Results

Reference standards were synthesised with an enantiopurity >99.8%. An achiral GC-MS method was validated and applied to seized herbal materials. SF-MDMB-PINACA (<1.0-91.5 mg/g herbal material) and AMB-FUBINACA (15.5-58.5 mg/g herbal material) were detected. EMB-FUBINACA and ADB-CHMINACA were also detected. For chiral analysis, a Lux Amylose-1 column was selective for SCRs with a terminal methyl ester moiety (AMB-FUBINACA and SF-MDMB-PINACA) and a Lux i-Cellulose-5 column for SCRs with a terminal amide moiety (AB-FUBINACA and AB-CHMINACA). As expected, the (S)-enantiomer predominated in all seized samples (93.6 to 99.3%) and (R)-AMB-FUBINACA was present to the greatest extent. For each enantiomer pair, the (S)-enantiomer was considerably more potent than the corresponding (R)-enantiomer. For CB1 the differences ranged from 6.11- to 114-fold depending on the enantiomer pair, and for CB2 1.55- to 63.5-fold. The relative potency of the enantiomers to the CB2 receptor is affected by structural features, the difference being more pronounced for compounds with an amine moiety than those with an ester moiety. All (S)-enantiomers had higher efficacy values at CB1 than the control JWH-018 as did the (R)-enantiomers of SF-MDMB-PINACA and AMB-FUBINACA suggesting that these compounds strongly and/or stably recruit βarr2 to CB1. Intrinsic metabolic clearance was estimated in vitro using human liver microsomes and human hepatocytes. SCRs with an ester-moiety had a faster clearance rate and shorter half-life (HLM: CLint 1.8 – 4.9 mL/min/g; half-life 5.7 – 6.6 min. HHeps: CLint 14.0 – 101 mL/min/g half-life 3.3 – 24.5 min) than compounds with an amide-moiety (HLM CLint1.8 – 4.9 mL/min/g; half-life: 15.1 – 48.9 min. HHeps 1.5-9.2 mL/min/g; half-life 38.7 – 332 min). The more prevalent (S)-enantiomer of AMB-FUBINACA had a faster clearance rate than the (R)-enantiomer.

Discussion

The methods described in this study are extendable to other chiral carboxamide-type SCRs (e.g 5F-MDMB-PICA and 4F-MDMB-BINACA) to aid pharmacological and toxicological assessment. The use of chiral profiling for tert-leucine (e.g MDMBs) and tert-leucinamide (e.g. ADBs) based compounds is expected to be of limited value, however valine methyl ester (‘AMB’ compounds) and valinamide (ABs) based compounds may show greater chiral variability. The use of SCRA enantiomers has helped further elucidate the SARs of this compound class and provides guidance for ‘prophetic’ synthesis and evaluation of SCRA analogues yet to appear on the market, but likely to do so.

Conclusions

The combination of achiral analysis, chiral analysis and a novel activity-based bioassay is a useful tool to assess the toxicological and pharmacological harms of a range of carboxamide-type SCRs. The calculation of an estimated intrinsic potency value for each seized sample helps to simplify the communication of risk related to SCRs containing samples although further studies into potential SCRA signalling bias and their influence on physiological effects are required.
Abstract ID 5
Fully automated determination of phosphatidylethanol 16:0/18:1 and 16:0/18:2 in dried blood spots.
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Aim
Direct alcohol markers are widely applied during abstinence monitoring, driving aptitude assessments, and workplace drug testing. The most promising direct alcohol marker was found in Phosphatidylethanol (PEth). The discovery of PEth extended the window of detection for direct alcohol markers in body fluids to several weeks. In human blood, the two most abundant PEth species are PEth 16:0/18:1 and PEth 16:0/18:2. PEth in liquid blood proved to be unsuitable for routine analysis, as it is unstable during storage and transportation above -80°C. Therefore, dried blood spot (DBS) sampling is preferred, as PEth can be stabilized due to the inactivation of enzymatic activity. Furthermore, sampling as DBS prevents the post-sampling formation of PEth, allows shipping without biohazard labeling in a standard envelope, and does not require any sample cooling. To facilitate and accelerate the determination of PEth in DBS, we developed a fully automated analysis approach.

Methods
The validated and novel online-SPE-LC-MS/MS method with automated sample preparation using a CAMAG DBS-MS 500 system reduces manual sample preparation to an absolute minimum, only requiring calibration (six-point calibration, 20-1500 ng/mL) and quality control DBS cards (20, 45, 1180 ng/mL). For the DBS, BioSample TFN filter paper cards from Ahlstrom were used. These cards contain special MS grade filter paper manufactured by using deionized water. Elution of the DBS was performed by using 2-propanol (40 μL). The analytes were trapped on a polar-RP column (20 mm × 2 mm, 4 μm) which was preconditioned with water containing 0.1% formic acid. Afterward, elution and chromatographic separation on a Luna RP-C5 column (50 mm × 2 mm, 5μm) were realized using gradient elution with A (30%water/70%acetonitrile containing 2 mM ammonium acetate (v/v)), and B (2- propanol). With the chosen setup the total runtime for each sample is about seven minutes, including photographic documentation, internal standard application, extraction, chromatographic separation, and instrumental analysis.

Results
During the validation process, the method showed a high extraction efficiency (>88%), linearity (correlation coefficient >0.9953), accuracy and precision (within ±15%) for the determination of PEth 16:0/18:1 and PEth 16:0/18:2. The two monitored PEth analogs could be baseline separated by liquid chromatography. A method comparison in liquid whole blood of 28 authentic samples from alcohol use disorder patients showed a mean deviation of less than 2% and a correlation coefficient of >0.9759. The comparison with manual DBS extraction showed a mean deviation of less than 8% and a correlation coefficient of >0.9666. Furthermore, no interferences were observed for blood samples from teetotalers. By the use of two internal standards, PEth 16:0/18:1-D5 and 16:0/18:2-D5, it was possible to compensate for potential matrix effects.

Discussion
The automated extraction approach proved to be fast and reliable for the determination of PEth samples. The method proved to be linear, accurate, and precise. In contrast to only analyzing the most abundant PEth species, PEth 16:0/18:1, the here presented approach has an unneglectable advantage: The quantification of the two most abundant PEth homologs allows confirmation of the result within the run. In abstinent subjects, both of the analogs are present at low concentrations; in AUD patients, on the other hand, both analogs are present at elevated concentrations.

Conclusions
The automated system, ideally suited for high-throughput analysis of PEth, provides a short turnaround time for large quantities of samples. In contrast to the manual extraction of PEth in DBS, no laborious sample preparation is required with this automated approach. This simplified and fast acquisition method may lead to a broader acceptance of PEth for routine analysis.
Abstract ID 164
Cocaine and related compounds in the hair of coca leaf chewers, coca tea drinkers and their comparison with cocaine addicts by RIA, UPLC-DAD-MS/MS.

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Aim
The chewing of coca leaves (Erythroxylum coca) and coca beverage infusion are common in the certain South-American region, with peculiar cultural and religious connotations. The “coqueo” abuse cases are not habitual in these communities. We have found few studies to the presence of related components of the coca leaf in pericranial hair from coca leaf chewers, coca tea drinkers and their comparison with cocaine addicts.

For this reason, we face this investigation in order to establish parameters that serve as comparison or confrontation to distinguish these three circumstances.

Methods
100 mg of pericranial hair from 100 consumers of coca leaf, 100 coca tea drinkers and 40 addicts to cocaine by inhalation were taken. Initially one came to the washing from the hair with dichloromethane and water, successively, at 37°C. The washing liquids were kept for later analysis. Later, the samples were digested with HCl 0.1 N to 50°C during 24 hours, and then filtrate and processed by SPE (Bond Elut C-18), retaken with 500µL of methanol. RIA methodology was applied by kit Cocaine Metabolite-Coat-A.Count-DPC previous conditioning. After these, UPLC-DAD-MS/MS was used, with special emphasis on cocaine (C), benzoylecgonine (BZ) and cocaethylene (CE), this last one found in joint ethanol ingestion and cocaine. The extracts were injected in an equipment Aquity UPLC (Waters), with column C18 (50 xs 2.1 mm id) & 1.8 um particle. Mobile phase: buffer potassium phosphate 20 mm, pH 7. ESI + and MRM acquisition. IS: Bz-d3; C-d3. DAD Detector (Waters). Bz & C.

LOD and LOQ: 0.05 ng/mg and 0.15 ng/mg.

Results
The BZ measurements by RIA gave for the three groups (mean): Infusion 20-30 ng / mg; chewed 50-60 ng / mg; Addiction 1-10 ng / mg CE was detected in some cases (2/100 coca chewers and ethanol)

BZ range in the three groups: 3.7-30 ng/mg of hair and for CE: 0.4-1.2 ng/mg. However, in five cases were observed C (3.7-30 ng/mg in cocaine addicts) higher than Bz for the two groups chewed and drink respecting to C addict.

Discussion
We will discuss the possible incidence of the route, type or variety of coca leaf consumed, in the detection in hair and origin of CE in chewing coca leaves persons (“coqueros”). Some authors have proposed the determination of other components of the Erythroxylum, such as Hygrine, cuscohygrine, in addition to establishing other component relationships. However, it requires more complex methodologies and standards that are not easily available. In our research we were able to establish over 240 cases a range of concentrations in hair that, in the first instance, allows us to distinguish whether the detected C or Bz come from drinking coca tea, chewing coca or ingesting cocaine in the form of powder.

Conclusions
We concluded that: 1) Initially, it is possible to distinguish between the three consumption groups according to the RIA data. 2) For tea drinkers and chewers, the Bz levels are much more significant than those of the C (> 1). 3) In the case of simultaneous consumption of alcohol and coca leaves, it’s possible to detect CE. 4) It should analyze another compound that allows differentiating coca leaf consume/ coca tea drinker.

Key words: chewing coca leaves, coca tea, cocaine addict, benzoylecgonine, cocaethylene, hair samples.

Acknowledgment: Fernando Galassi from UNNE University for RIA data.
Abstract ID 203
Workplace drug testing in Australia – dealing with prescription and non-prescription medications, dilute urine specimens and attempted adulteration.
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Aim
On-site drug screening is a valuable tool in the maintenance of a safe workplace. Workplaces often require support to interpret test results that are non-negative for drug classes of common prescription and non-prescription medicines. They also require assistance to manage specimens that are dilute (urine) or potentially adulterated (oral fluid and urine). In these cases, workplaces face a loss of productivity associated with workers stood down from duties pending further investigation. This must be balanced against the potential risk of a worker remaining on duty when drug-affected. This paper describes strategies that meet the needs of workplaces to manage these conflicting demands.

Methods
Anonymised data from onsite drug screening tests, and the laboratory confirmed outcomes were examined. Overview data for urine and oral fluid specimens covered July 2016 to June 2017. Codeine-confirmed data were collated from February 2017 to January 2018 (prior up-scheduling codeine-containing products) and compared with February 2018 – January 2019. Data were from a range of industry and government sectors. Data were presented by year and by monthly trend analysis, and with targeted seasonal analysis. Subset data analysis was performed for a representative industry (aviation) to examine the rate of dilute urine specimens requiring retest.

Results
Workplace data for 2016-2017 (23,454 tests) revealed 0.40% of oral fluids and 1.25% of urines were confirmed positive for illicit drug use (0.81% overall). 0.47% of oral fluids and 4.23% of urines were confirmed positive for medications (2.08% overall) with codeine representing 25% to 80% of detections, indicating that the potential for business down-time associated with medications such as codeine significantly affects productivity.

Comparison of two Government agencies with high rates of codeine detections showed that prior to up-scheduling of codeine, 229 of 6,641 tests (3.45%) were confirmed positive for codeine, whereas 88 of 7,667 tests (1.15%) were codeine positive after up-scheduling. The cold and flu season winter months (June-August) represented the bulk of detections. Winter season data for four industries (aviation, government, manufacturing, public transport and transport logistics) showed the rate of codeine positive samples fell from 1.03% (69 of 6,730 tests) to 0.21% (16 of 7,641).

Dilute urine specimens (falling out of range for two dilution integrity tests) were found in 2% (of 1469) of urine specimens from a representative industry group. Of these, half were found to be dilute at the point of collection. These dilute specimens required a retest to provide a valid urine specimen. Urine specimens that were dilute were generally negative for all drug classes tested. Workplace managers viewed these detections as a burden on productivity, and retesting these workers was a significant cost to business with little return in terms of safety.

Discussion
Workplace drug testing contributes to a safe workplace. On-site tests are designed to rapidly screen workers, allowing the majority of workers who are drug free to continue duties with only minor disruption to productivity. Workers who screen non-negative for drugs must submit to laboratory confirmatory testing. Pending laboratory test results, workers are generally stood down from duties.

In many cases, the on-site drug test may detect a drug consistent with declared medications (e.g. codeine). Standing down these workers may be a conservative approach to safety. We recommend that workers assessed as unimpaired, may perform alternate duties whilst awaiting confirmation results. This allows businesses the flexibility to manage personnel with less disruption.

Similarly, urine may be dilute or require further investigation for other reasons. If on-site tests are negative, then standing down workers may be undesirable. Workers assessed as unimpaired may remain at work pending confirmation test results, and may also not require additional testing.

The small proportion of urines identified as potentially adulterated or substituted may be managed under a targeted testing regime. Anecdotally, we have seen an increase in oral fluid specimens that are difficult to confirm by laboratory analysis (suggesting workers are submitting sputum rather than oral fluid or saliva). We recommend drug test personnel identify these specimens by their appearance (colour, viscosity, odour) at the point of specimen collection and designate them as “failure to provide a specimen”. In these cases, as with refusal to test, this is a presumption of a confirmed positive drug test and is managed under existing workplace drug testing policies. In our experience, workplace managers valued these assessments as a positive way to identify donors attempting to evade detection.

Conclusions
Managing medication-related and dilute or adulterated specimens in workplace drug testing programs in Australia remains a significant issue. Greater effort is needed to inform and educate managers to recognise and respond to these events to ensure workplace safety, and toxicology advice including commentary on the quality of drug testing specimens is essential. The significant reduction in codeine detections following up-scheduling of these medicines in Australia has resulted in benefits for workplaces including reduced productivity down-time and lower direct costs of laboratory confirmations and worker retests.
Abstract ID 298
Incidence of drugs of abuse within the Australian Workforce subject to random drug testing.

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Self

Aim
Previously little direct evidence has been available for the overall incidence of drugs of abuse in the Australian workplace other than self-reported surveys, which may not be totally reliable given the circumstances involved in declaring drug use on-site.

This study analyses 432,782 workplace drug tests performed in Australia from 2017 to 2018, utilising both oral fluid and urine testing (typically performed to the respective Australian Standards AS4760 and AS4308).

Methods
The data is geographically diverse (collected from all states and territories in Australia), industry/sector diverse (mining, transport, constructions, logistics, aviation and utilities companies predominate), utilises a wide range of screening technologies (laboratory based urine immunoassay, 5 separate on-site urine devices and 8 separate oral fluid screening devices), is collected via a variety of mechanisms (Safework Laboratory trained collectors, employer self testing, referrals from other accredited and/or non accredited collection agencies), and is specific for workplace testing only (no data has been collected from any clinical source or drug rehabilitation program).

Results
Overall positive screening results in urine (for all AS4308 listed drugs) in 2017 and 2018 respectively are 7.9% and 7.3%, with 76.6% and 77.1% of those screening results being confirmed by LC/MS. Overall positive rates in oral fluid (for all AS4760 listed drugs) in 2017 and 2018 respectively are 1.3% and 1.1%, with 61.8% and 61.6% of those results being confirmed by LC/MS. Further data on screening positives by individual drug type and incidence, as well as false positive results both by drug class and matrix are also calculated.

Discussion
Conclusions
Oral fluid and urine matrices tested according to the relevant Australian Standards vary considerably in both the incidence of detection of various illicit drugs, but also in the rate of false positives for on-site testing devices overall. Consideration of these differences may be relevant in choice of testing matrix for any organisation undertaking a testing protocol.
Abstract ID 326
Deep Learning as a tool for classification of biological samples measured by HRMS: Detection of adulterated urine samples and cosmetically treated hair samples.
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Aim
Adulteration of urine samples or cosmetic treatment of hair (e.g. bleaching and dyeing) is a typical problem in daily forensic casework. Different procedures are available to uncover such urine or hair samples (e.g. urine dip tests or detection of markers such as PTCA in hair samples). Ideally, a drug testing workflow would include drug detection with simultaneous screening for adulteration attempts within the very same run. The same applies for an efficient workflow in hair analysis. With liquid chromatography coupled to high resolution quadrupole time-of-flight mass spectrometry (LC-HR-QTOF) with data-independent acquisition (DIA) becoming more and more a standard in forensic toxicology, the resulting data files contain much more data than just the typical target analytes. The aim of this study was to assess whether a neural network, which had been created by us, can be trained to read those HRMS data and learn to distinguish between adulterated and untreated urine samples and between treated and untreated hair samples in a binary classification deep learning approach.

Methods
Urine samples (n=100) were treated with five different adulterants (i.e. KNO2, H2O2, NaOCl, I2 and pyridinium chlorochromate (PCC), 20 urine samples per adulterant). Additionally, 100 untreated urine samples were prepared as control. For the hair samples, 21 treated with a bleaching agent and 21 untreated hair samples were prepared. All samples were measured with LC-HR-QTOF (Sciex TripleTOF 6600, Baden, Switzerland). A neural network (NN) was built in R (R x64 3.5.1 and R Studio 1.1.463) using Keras, a high-level NN application programming interface (API) capable of running with TensorFlow as the back-end. For both experiments, a labeled training set, including samples from both categories (treated/bleached vs. untreated) was used to train the feedforward NNs. Afterwards, the NNs were tested to predict the category of new samples from a test set, not seen during training.

Results
For the urine test samples, some adulterants were correctly predicted with a rate of 78% or others with up to 94%. By training the NN with all samples (100 treated and 100 untreated samples), a test set including 20 samples (10 treated with the 5 adulterants, respectively, and 10 untreated) was correctly categorized with a rate of 70% on average. For the hair samples, the correct prediction of the test set including 6 samples (3 bleached and 3 untreated samples) was possible with a rate of 94% after training on a training set including 36 samples (18 samples for each group, bleached and untreated).

Discussion
It could be shown, that NNs can be used for such triage purposes. In the case of urine samples, correct prediction was possible in up to 94% of the cases. For the hair samples, the correct prediction rate was in a similar range. Both approaches (for urine and hair) showed results well suited as a method in a routine laboratory workflow to get information on whether or not a sample was manipulated.

Conclusions
Deep learning promises a huge potential for automatic handling of big data generated by high resolution mass spectrometry in analytical laboratories. The created NN used routine HRMS data to reliably uncover manipulated urine or hair samples. Thus, this approach may be an interesting alternative for time- and resource-consuming methods in routine laboratories.
Abstract ID 330  
**Prevention of False Negativity with Creatinine Normalization.**  
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**Aim**
Creatinine measurement is important for screening, semi-quantitative screening analyses for patients, probationers and workplace drug testing cases in forensic toxicology field. Urinary creatinine concentration serves as an indicator of urine dilution. Diluted urine samples affect the illegal substances concentrations, remain under the cut-off values and these results are interpreted as false negative. In this study, we aimed to choose the most appropriate method to prevent false negativity by examining the values obtained from formulas including creatinine concentrations with drug screening test.

**Methods**
In this study, all cases reports that applied our laboratory for urine drug screening were examined between 2015-2018 (n=25,630). Screening analysis were performed for THC-COOH, amphetamine and derivatives, opiate, benzodiazepine, cocaine and synthetic cannabinoids (JWH-018, JWH-073, AM-2201, UR-144) and creatinine (CR) with Olympus AU400 (Thermo Scientific assays). Urine dilution was defined by European Workplace Drug Testing in Urine Guideline. All cases drug screening test results, CR concentrations, age, sex, urine collection time information’s and confirmation analysis results were evaluated. Statistical analysis was performed using SPSS 25.0 program.

**Results**
25,630 cases drug screening results were investigated and mean CR value was calculated 143.71±83.68 mg/dL. Annual average of CR value was calculated for each year, respectively, 153.23±87.66 (2015), 152.84±87.33 (2016), 135.18±78.10 (2017) and 135.58±80.35 (2018) mg/dL. When the CR values of male and female cases were investigated, there was a significant difference between CR and gender (p<0.05, male mean CR: 145.17±83.75, female mean CR: 123.46 ±80.07 mg/dL). When the age values of all cases were investigated; the age mean was 27.08±9.85 mg/dL. However, no significant correlation was found between age and CR levels (r = -0.082, p<0.01, N=25,630). When the age values grouped by 10 years, there was a significant difference between age groups and CR mean values (p<0.05, minimum 64.46±36.68 mg/dL (age group:0-9); maximum:149.41±85.32 mg/dL (age group:10-19)). Also, there was a significant difference between urine amposes collection time and CR mean values (p<0.05, minimum:84.65±68.28 mg/dL (at 06:00-06:59), maximum:149.85±85.07 mg/dL (at 11:00-11:59)).

The urine CR level of 1,062 cases was found between 5.6-22.6 mg/dL and reported as ‘sample diluted’. 17.36-20.43% for THC-COOH, 4.70-6.33% for amphetamine and derivatives, 0.54-0.90% for opiates would have been reported positive for screening, after normalization with the lowest and highest mean creatinine values to dilute samples. Specimens defined as positive, after confirmed by GC-MS and reported as positive.

**Discussion**
Creatinine is a waste product formed by the spontaneous, essentially irreversible dehydration of body creatine and creatine phosphate from muscle metabolism and it is cleared from the body through the kidney primarily by glomerular filtration. Urinary creatinine has been used to estimate biomarker excretion rates because it is excreted at a reasonably constant rate, and thus it is useful for normalizing spot urine samples within demographic groups. The normalization of biomarker excretion rates among different demographic groups may be compromised due to reasons such as muscle mass, gender, age, diet and kidney function differences in creatinine excretion (Xia, 2014). In the study reported by Price (2013), the mean creatinine concentration was calculated as 119.04 mg/dL and this value used for normalization procedures to use linear ratio. At the same study said that, a survey reported the mean urinary creatinine concentration as 130.40 mg/dL. The study found that, the postnormalization negative group urine creatinine concentration is identical to the higher creatinine level group. However, the results between positive and false negative data were determined according to the calculated different creatinine mean values obtained in our study.

Specific gravity and pH parameters are the other important factors for urine dilution. Some studies have indicated a strong correlation between specific gravity and creatinine. Another study found that, creatinine is the more influential variable in accounting for urine dilution (Xia, 2014). In addition, many urine substance normalization studies are used to ratio format to normalize analyte quantification for specimen concentration. The normalization process involves dividing the concentration of the analyte of interest by the creatinine concentration obtained in the same urine sample (Hou, 2012). However, this approach is insufficient to apply the cut-off value in forensic toxicological analyzes. Therefore, different statistical methods should be developed.

**Conclusions**
When evaluating drug testing reports, individual characteristics such as gender, age, weight and height, also urine sample collection time are considered. On the other hand, there is no published local creatinine value for spot urine samples in many countries and in also Turkey, with this study, these shortcomings will be eliminated. According to the social structure, appropriate normalization models should be developed and should be included in the guidelines.
Abstract ID 403
Recreational vs. medical use of amphetamine-type phenethylamines: Enantioselective analysis of metabolites in urine samples.
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Aim
The enantiomeric profile of thirteen structurally related phenethylamines was determined in urine after the suspected consumption of amphetamine, methamphetamine and pseudoephedrine. Metabolic connections were evaluated with a focus on stereochemistry.

Methods
Authentic urine samples from forensic cases (n=116) and from a controlled self-administration study with a medical pseudoephedrine preparation (n=38) were prepared by automated solid phase extraction (Aspec® GX-274, Gilson, Middleton, WI, USA) using bifunctional CHROMABOND® Drug cartridges (Macherey-Nagel, Düren, Germany). Chromatographic separation was carried out on a Nexera X2 UHPLC system with a chiral column (Lux® 3 µm Amp, Phenomenex, Aschaffenburg, Germany). A 26 minute gradient with an alkaline mobile phase A and a methanolic mobile phase B was applied. A triple quadrupole mass spectrometer (QTRAP® 5500, Sciex, Darmstadt, Germany) operated in ESI+ and MRM mode was used as a detector. The method was fully validated. Metabolic pathways were evaluated based on the enantiomeric profile of (R)/(S)-amphetamine, (R)/(S)-methamphetamine, (1R,2R)/(1S,2S)-pseudoephedrine, (1R,2S)/(1S,2R)-ephedrine, (1R,2S)/(1S,2R)-norephedrine, (R)/(S)-cathinone, and (1S,2S)-norpseudoephedrine (cathine).

Results
Samples with assumed consumption of amphetamine (n=65) showed both enantiomers with (R)/(S)-amphetamine ratios always greater than one. The rate of detection for (1R,2S)-norephedrine, (1S,2R)-norephedrine and cathine in samples with low amphetamine concentrations ≤ 200 ng/mL was 83%, 20%, and 34%. In samples with high amphetamine concentrations > 200 ng/mL (1R,2S)-norephedrine, (1S,2R)-norephedrine and cathine were detected in 100%, 90% and 83% of the samples. Furthermore, low concentrations of (R)- and (S)-methamphetamine were detected in 67% and 50% of the samples with high amphetamine concentrations. In nine samples only (S)-amphetamine was detected in concentrations between 650 and 25,000 ng/mL, implicating the uptake of a medicinal amphetamine preparation or a precursor drug (e.g. lisdexamphetamine). Next to (1R,2S)-norephedrine (100%), and cathine (66%), small concentrations of (S)-methamphetamine (1.1-7.2 ng/mL) were detected in the four samples with the highest amphetamine concentrations. Assumed methamphetamine consumption (n=26) is typically characterized by the presence of enantiopure (S)-methamphetamine and its main metabolite (S)-amphetamine. (1S,2S)-Pseudoephedrine, (1R,2S)-ephedrine, (1R,2S)-norephedrine, and cathine were found in relatively low concentrations. In samples after controlled (1S,2S)-pseudoephedrine self-administration (Rinoral® tablets) cathine, (1R,2S)-norephedrine and (R)- and (S)-cathinone were detected.

Discussion
In forensic cases information on the actual composition of consumed drugs is not always valid. Therefore, a differentiation between co-consumption and metabolic formation of a detected substance often remains tentative. Nevertheless, the increasing detection of cathine and (1S,2R)-norephedrine next to (1R,2S)-norephedrine in samples with high amphetamine concentrations indicates their metabolic formation. The presence of both methamphetamine enantiomers – which is untypical after methamphetamine consumption – in such samples suggests a metabolic or artefactual formation by methylation. This is also supported by the findings of (S)-methamphetamine in samples after suspected consumption on enantiopure (S)-amphetamine. Self-administration of a medical (1S,2S)-pseudoephedrine preparation caused findings of cathinone, which is a controlled substance in many countries. The results strongly indicate that cathine is a metabolite of (S)-amphetamine. A formation of racemic methamphetamine from racemic amphetamine is suggested as a minor metabolic pathway. The additional consumption of (S)-methamphetamine can be clearly distinguished by enantioselective analysis. The self-administration study showed that cathinone findings can be caused by the uptake of medical (1S,2S)-pseudoephedrine preparations. Therefore, cathinone always has to be determined simultaneously with pseudoephedrine.

Conclusions
The observations made in this study should rise awareness for the complex pathways of phenethylamine metabolism and the inherent difficulties in interpreting analytical results. When evaluating phenethylamine positive cases, a possible metabolic formation of the detected compounds from a related substance should always be considered. Comprehensive and chiral analysis can facilitate the interpretation.
Abstract ID 552
Detection of delta-8-THC-COOH in urine samples and its implications in workplace drug testing.
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Aim
Delta-8-THC is a psychoactive cannabinoid produced in small quantities by Cannabis sativa. This compound has been studied for its inhibitory action on cancer cells1 anti-emetic effects in pediatric cancer patients2. Recently delta-8-thc enriched extracts and dabs have been marketed for their milder psychoactive effects as compared to delta-9-thc only products. Due to its similar structure to delta-9-thc, this molecule and its metabolites pose a potential interference in the analysis of urine specimens. In this study, we developed a method that resolved the delta-8 and delta-9-THC metabolites and applied it to over 1,100 human urine specimens from court-ordered monitoring programs in the U.S.

Methods
Samples were prepared by taking a 250 µL aliquot of the calibrator, control, or specimen, adding 25 µL of internal standard, 50 µL of 3 N KOH, hydrolyzing at 37°C for fifteen minutes. After hydrolysis, the samples were neutralized with 12.5 µL of glacial acetic acid, and precipitated with 350 µL of acetonitrile prior to centrifugation for 3 minutes at 15,000 g. A 50 µL aliquot of the supernatant was added to 750 µL of a 70:30 DI water:acetonitrile solution containing 0.01% acetic acid. A multiplexed Shimadzu Prominence HPLC system connected to a Scieux 4000 Qtrap was used with negative mode with three transitions monitored for the cannabinoid metabolites 343.1 > 299.3, 343.1 > 245.0, and 343.1 > 190.9 and one transition monitored for the deuterated internal standard 346.2 > 248.3. Mobile phases were 5 mM ammonium formate in de-ionized water (A) and 5 mM ammonium formate in methanol (B). The 3.5 minute gradient started at 80% B for 0.8 minutes ramped to 100% B over 0.7 minutes and held at 95% B for 1 minute prior to a 1 minute re-equilibration at 80% B. The analytical column was a 50 x 4.6 mm 2.7 m Supelco Ascentis Express with a 5 x 4.6 mm guard column.

Results
Approximately 3 % of the specimens tested contained the delta-8-THC-carboxy metabolite, and all contained the delta-9-THC-carboxy metabolite, with the delta-8-THC metabolite eluting 0.1 min earlier than the delta-9-THC metabolite. In all but four specimens, the delta-9-THC metabolite concentration was higher than that of the delta-8-THC metabolite. Concentrations of the 11-nor-delta-8-THC-carboxy varied from 8-459 ng/mL with little correlation to the 11-nor-delta-8-THC-carboxy concentration.

Discussion
Laboratory directors should be aware of the presence of this metabolite in human urine specimens, and its implications in results reporting. For example, laboratories could be reporting false negatives if the presence of delta-8-THC-carboxy causes specimens to fail retention time reporting criteria. Additionally, the presence of the delta-8 metabolite could falsely elevate delta-9-THC-carboxy levels if the two metabolites co-elute.

Conclusions
Delta-8-THC and Delta-9-THC carboxy metabolites were successfully resolved and quantitated in over 1,100 specimens with the Delta-8-THC-carboxy being found in 3% of the specimens. The presence of delta-8-THC carboxy metabolite in urine specimens poses a problem for workplace testing laboratories which should be addressed in their testing methodologies and reporting guidelines.


Abstract ID 6
Increment in concentrations of Ethanol in Blood and Vitreous Humor found in the last three years in Argentina.
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Aim
This abstract's objective is to report that in Argentina, in postmortem samples, in which no stimulant substances were present, we have observed a sustained increase in quantities of Ethanol, both in blood and vitreous humor, and where the expected clinical state reported in bibliographical tables don't coincide with the circumstances prior to the deaths.

Methods
To 10 ml head-space vials we add 0.1 ml of sample and 1,0ml of t-buthanol internal standard solution. Each sample is duplicated and, for most cases, we receive from the medical examiner, blood and vitreous humor.

We run the samples with an 8-level curve (0.1 g/L to 5.0 g/L) and each batch includes a negative control and two positive controls of different concentrations.

Our method has been validated following the criteria of SWGTOX (Scientific Working Group for Forensic Toxicology) - Standard Practices for Method Validation in Forensic Toxicology.

Equipment and materials:
- HS-FID/GC (DANI) with two columns (BAC-1 30m x 0.32 mm x 1.80 and DB ALC-2 ZB 30m x 0.32 mm x 1.20), both of them used during the validation.
- Callibrated pipettors

Results
In the following tables we show the number of study cases (that we'll name "Cases X"), the increase of these cases during the last three years and the circumstances prior of death of the subjets in these cases.

Table 1:
2016:
Total nº of cases: 3977
Positive cases: 260
Cases "X": 3
% Cases "X"/Positive cases: 1.15
% Cases "X"/Total nº of cases: 0.08
2017:
Total nº of cases: 3745
Positive cases: 134
Cases "X": 6
% Cases "X"/Positive cases: 4.47
% Cases "X"/Total nº of cases: 0.16
2018:
Total nº of cases: 3604
Positive cases: 241
Cases "X": 11
% Cases "X"/Positive cases: 4.56
% Cases "X"/Total nº of cases: 0.31

Table 2: Cases "X"
2016
- M/62 - Suicide (Jumped out of a window) : Blood: 3.0 g/L Vitreous Humor: 3.0 g/L
- M/51 - Driving a motorcycle : Blood: 3.0 g/L Vitreous Humor: 3.1 g/L
- M/24 - Driving a motorcycle: Blood: 3.0 g/L Vitreous Humor: 2.9 g/L

2017
- M/31 - Suicide (Hanged himself) : Blood: 3.2 g/L Vitreous Humor: 2.8 g/L
- M/45 - Walking on the street: Blood: 3.7 g/L Vitreous Humor: 4.2 g/L
- F/60 - While having dinner during commercial flight: Blood: 3.4 g/L Vitreous Humor: 3.4 g/L
- M/65 - Walking on the street: Blood: 4.0 g/L Vitreous Humor: 4.4 g/L
- M/56 - Having an argument at home : Blood: 5.0 g/L Vitreous Humor: 5.0 g/L
- M/46 - Having dinner at home : Blood: 3.5 g/L Vitreous Humor: 4.4 g/L

2018
- M/61 - In the shower : Blood: 3.1 g/L Vitreous Humor: 3.3 g/L
- M/46 - Walking on the street : Blood: 3.8 g/L Vitreous Humor: 3.7 g/L
- M/40 - During a street fight : Blood: 3.4 g/L Vitreous Humor: 3.1 g/L
- M/40 - During a family Dinner : Blood: 3.8 g/L Vitreous Humor: 3.8 g/L
- M/63 - Walking on the street : Blood: 3.8 g/L Vitreous Humor: 3.8 g/L
- F/56 - At a party : Blood: 6.0 g/L Vitreous Humor: 7.0 g/L
- M/62 - Walking on the street : Blood: 4.8 g/L Vitreous Humor: 4.0 g/L
- M/25 - Street fight : Blood: 3.4 g/L Vitreous Humor: 3.8 g/L
- M/43 - During a family dinner : Blood: 3.4 g/L Vitreous Humor: 3.4 g/L
- M/65 - Obstruction of airway during dinner : Blood: 3.1 g/L Vitreous Humor: 3.2 g/L
- F/69 - Walking on the street: Blood: 3.3 g/L Vitreous Humor: 3.3 g/L

Table 3: Averages
2016
Average Blood concentration: 3.0
Average Vitreous Humor concentration: 3.0
2017
Average Blood concentration: 3.8
Average Vitreous Humor concentration: 4.0
2018
Average Blood concentration: 3.8
Average Vitreous Humor concentration: 3.9
Discussion
From the data shown on Table 1, we observe an increment in the number of study cases included in the positive samples and an even more remarkable increment when compared with the total number of postmortem samples processed each year. There is a good correlation between the amount of ethanol found in blood and vitreous humor of every sample (Table 2). We observe that the average ethanol concentration in blood and vitreous humor is larger in 2017 and 2018 than in 2016 (Table 3). Since the average concentrations are more than 3.0 g/L these subjects are expected to have been unconscious, asleep or in a coma and not in the circumstances prior to the deaths that are detailed in Table 2.

Conclusions
We report, in our country, an increase in the number of cases in which the circumstances prior to the deaths don’t coincide with the expected clinical state that is shown in the bibliographical tables for the high concentrations of ethanol we have found in these samples. We suspect, given that Argentina is the first ethanol consumer in LatinAmerica, that these findings have to do with an increase of tolerance. In every case reported, the subjects were active and not unconscious, asleep or in stupor or coma (Molina and Hargrove 2019 - Garriott 2015)
Abstract ID 17

Death of a newborn in a nursery: is hydroxyzine involved?

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Aim
Discuss a potential toxic cause of death of a newborn exposed to hydroxyzine via placenta transfer, while the drug was found in blood at a therapeutic concentration (as per reference to an adult), using hair analysis results from both the newborn and the mother.

Methods
The mother was treated with Atarax (hydroxyzine) and Spasfon (phloroglucinol) 48 hours before delivery, after a false labor. Following an emergency cesarean section in a context of a prolonged slow heart rate with no recovery, maternal fever of 39 °C, and lactate levels of 6.2 mmol/L, the child D was delivered in a cephalic presentation (birth weight: 3084 g, Apgar 03/08/10). Due to respiratory distress syndrome, the newborn was transferred to the neonatal intensive care unit and underwent noninvasive ventilation until its 10th hour of life. After 12 hours of life, the baby showed good hemodynamic status, normal capillary blood glucose, a normal neurological examination, and took easily the bottle. During this initial period, the biological tests highlighted neonatal anemia with 13.3 g/L of hemoglobin, a suspected maternal-fetal infection with a cord PCT concentration of 0.43 ng/ml and a CRP concentration of 3.9 mg/L. Considering its good adaptation, the newborn was transferred to the postnatal unit on D1 of life weighing 2930 g and receiving dual antibiotic treatment (amoxicillin and gentamicin). However, the next day, the baby was discovered deceased in its cradle in the nursery.

The autopsy did not reveal any visceral malformation or visible macroscopic signs of infection. No traumatic injury that could explain the death was observed. The results of the blood bacterial culture were negative for both the newborn and the mother. To document the cause of death, a full toxicological investigation was requested by the Judge in charge of the case.

In adults, therapeutic blood hydroxyzine concentrations range from 50 to 90 ng/ml, with cetirizine in the range from 20 to 400 ng/ml. Drug sequestration by the newborn has to be discussed, given hydroxyzine was administered to the mother 48 hours before delivery. Hair analysis should be read as repetitive administration, but there is currently no reference data for interpreting hydroxyzine and cetirizine data. However, these results are likely not representative of contamination.

Discussion
The child’s blood concentration shows recent exposure. In the mother’s hair: the presence of hydroxyzine and its metabolite, cetirizine, over the entire segmental analysis of 4 X 3 cm indicates repeated exposure to Atarax over the period studied.

In child’s hair: the concentrations measured suggest that the child was repeatedly exposed to Atarax. In-utero exposure may be possible (incorporation into the hair as early as the last trimester of pregnancy).

The absence of a controlled study does not make it possible to establish the dose nor the frequency of exposure to Atarax. Transplacental passage of Hydroxyzine and cetirizine occurred (passive transport).

The concentration of cetirizine in the child’s hair was higher than the concentration of hydroxyzine. The transplacental passage of cetirizine could be higher than hydroxyzine (active transport).

Conclusions
This observation highlights repeated exposure of the mother to hydroxyzine (Atarax). The presence of this molecule in the newborn indicates a maternal-fetal transfer. Atarax can trigger cardiac rhythm disorders (torsades de pointes) and can cause QT interval prolongation. In the hair of the newborn, the ratio (< 1) of parent hydroxyzine to its carboxylated active metabolite is unusual. The direct attribution of hydroxyzine and its metabolite in the death of the child remains very difficult to establish and will be deeply discussed during the presentation. Should Hydroxyzine (Atarax) continue to be prescribed during pregnancy? Should it be used before delivery?
Abstract ID 25
Total mercury determination by TDA AAS in body fluids and tissues for forensic purposes.

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Aim
Mercury (Hg) is a heavy metal with high toxicity. One of the newer techniques for determining trace amounts of total mercury in various materials, including biological samples, is thermal decomposition, amalgamation, and atomic absorption spectrometry (TDA AAS).

The aim of the study was evaluation of the possibility of using the TDA AAS method (Direct Mercury Analyzer DMA-80) to determine reference levels of total mercury in biological post-mortem samples for the forensic purposes.

Methods
The limits of detection for Hg were 0.10 and 0.20 µg/L (nickel and quartz boats, respectively). The working range of the calibration curve was at least from 0.6 to 200 ng Hg/mL; the intra-day precision in samples (RSD) – in the range of: 1.66 – 6.86% (blood), 0.82 – 1.47% (urine), and 2.01 – 3.44% (hair); the inter-day precision (over 8 days): 2.51%, and 2.50% (blood spiked with 2.5 and 10 ng Hg, respectively), 5.10%, and 3.16% (urine spiked with 2.0 and 6.0 ng Hg, respectively). The accuracy (as mean value of relative error) determined on the basis of the study of reference materials of blood (Seronorm Trace Elements Whole Blood L-1, L-2, L-3), urine (Seronorm Trace Elements Urine, Urine L-2), hair (Human Hair NIES CRM No. 13), and liver (Bovine Liver 1577c) was: 1.25% (blood), 0.50% (urine), 0.86% (hair), and 5.97% (liver); recovery of 2.5 ng Hg (blood): 93 – 97%.

Results
The method was used for the determination of mercury in 76 samples of various biological matrices, including samples of whole blood, urine, hair, bile and vitreous humour obtained from autopsy cases relating to inhabitants of Southern Poland who had not been poisoned and had not been exposed environmentally or occupationally to mercury or other heavy metals. Mercury concentrations in post-mortem blood (n = 24) were in the range: 0.61 – 12.4 ng/mL (median 3.02 ng/mL); urine (n = 12): 0.16 – 2.19 ng/mL (median 0.81 ng/mL); hair (n = 14): 0.08 – 0.53 ng/mg (median 0.22 ng/mg); bile (n = 12): 1.15 – 7.11 ng/mL (median 2.41 ng/mL); and vitreous humour (n = 13): 0.22 – 1.01 ng/mL (median 0.47 ng/mL). Concentrations of Hg in blood, urine, and internal organs samples obtained from young children (<3 years) and adults in several autopsy cases, were also reported.

Discussion
Conclusions
In conclusion, the method is suitable and convenient for the purposes of forensic toxicology examinations.
Abstract ID 27
Quantification of U-47700 and its metabolites: N-desmethyl-U-47700 and N,N-didesmethyl-U-47700 in 12 autopsy blood samples employing SPE/LC-ESI-MS-MS.
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Aim
The paper presents a report on the comprehensive assessment of a novel synthetic opioid (NSO) termed U-47700, and its two metabolites: N-desmethyl-U-47700 and N,N-didesmethyl-U-47700 in autopsy blood samples taken from 12 cases of fatal poisonings.

Methods
The analysis of the examined samples was based on the solid-phase extraction/liquid chromatography–electrospray ionization-tandem mass spectrometry method, which was developed in the validation process. In the quantitative analytical studies, deuterium analogues were used, namely U-47700-d6 and N-desmethyl-U-47700-d3.

Results
The parameters of the method were determined, including the limits of quantification on the 1 ng/mL level, calibration curves ranging from 1–1000 ng/mL, intra-assay precision and accuracy of 1.1–20.2% and -18.9–9%, respectively, inter-assay precision and accuracy of 2.9–13.0% and -11.4–3.3%, respectively. The matrix effect and the extraction efficiency were formed at the levels of 54.0–119% and 53.0–118%, respectively. The parent substance and its metabolites in blood samples have been shown to be relatively stable under various conditions within the 21-day study. The concentration levels demonstrated in the analyzed blood samples were: U-47700 in the range of 83–24000 ng/mL, N-desmethyl-U-47700 in the range of 2.0–7520 ng/mL and N,N-didesmethyl-U-47700 in the range of 18–1947 ng/mL.

Discussion
U-47700 as a synthetic opioid is a μ-receptor agonist, the action of which is manifested by a reduction in the sensation of pain and a reduction of emotions associated with pain, such as anxiety, fear and a sense of danger. The particularly dangerous side effects of opioid receptor agonists include the development of tolerance and addiction, and respiratory depression that can lead to death. Although U-47700 was synthesized at the end of the last century as an opioid analgesic drug class in an attempt to develop a non-addicting analgesic, it has appeared only in recent years on the drug market, gaining popularity among drug users as a legal alternative to heroin. U-47700 is available on the Internet in a powdered form which is usually taken through nasal inhalation or ingested in a capsule form. In 2016, U-47700 was sought with a very high interest and was highly visible on the Internet, suggesting a new trend on the horizon for synthetic opioid abuse.

Conclusions
We have presented, for the first time, a report on the comprehensive assessment of an NSO termed U-47700, and its two metabolites N-desmethyl-U-47700 and N,N-didesmethyl-U-47700 in autopsy blood samples collected from 12 cases of fatal poisonings. For this purpose, we developed a sensitive and high-selective SPE/LC–ESI-MS/MS method in the validation process. The values quantified by the present method for U-47700 and its two metabolites in human blood samples seem very useful to confirm the cause of death and to estimate the antemortem-to-death interval after consumption of U-47700.

Toxicological findings of deaths associated with U-47700, which were subject of the present study, originating from expert’s medico-legal opinions issued in the years 2016-2017. The concentrations of the drug in the samples of blood were in a wide range of 83-24000 ng/mL. In all the examined cases, apart from U-47700, other xenobiotics were detected, including alcohol in four instances, synthetic cannabinoids in four, medications in one, phytocannabinoid, and synthetic cannabinoids, synthetic cathinone and medications in one case, and synthetic cathinone and medications in two cases. In the presently discussed cases, owing to employing the highly selective and sensitive method, apart from U-47700, the investigated blood samples showed the presence of N-desmethyl-U-47700 in the concentration range of 2-7520 ng/mL and N,N-didesmethyl-U-47700 in the concentration range of 18-1947 ng/mL. Their concentration values in the blood samples as compared to that of the precursor were variable, without showing a discernible trend. It could be also hypothetically assumed that they can demonstrate biological activity, similarly as desmethyl derivatives of numerous xenobiotics, which may significantly affect the toxicity of U-47700.
Abstract ID 31
Screening for synthetic cannabinoids should not be limited to blood samples - considerations supported by case of poisoning with AMB-FUBINACA and EMB-FUBINACA.

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Aim
Synthetic cannabinoids are currently the largest group of new psychoactive substances. Those that have been subjected to legal control are replaced by newer uncontrolled substances, which causes constant and dynamic changes to the drug market. The popularity of this group of compounds is supported by the number of many serious poisonings, including fatalities. Some of the most recent synthetic cannabinoids that have appeared on the ‘legal highs’ market are AMB-FUBINACA and EMB-FUBINACA. This presentation concerns the toxicological findings in a fatal case, in which these new synthetic cannabinoids were detected and quantified in post-mortem materials. A 27-year-old man was found dead on a bed in an apartment. As a result of autopsy, congestion of internal organs, pulmonary edema and left-sided pleural adhesions were found. The autopsy materials (blood, urine, liver, kidney, stomach, intestine, lung and brain) were collected for further toxicological analyses.

The aim of the presentation is to answer the question: does the absence of synthetic cannabinoids in the blood exclude fatal intoxication with these compounds?

Methods
The autopsy materials were analysed for the presence of a wide range of prescription drugs and toxic substances including new psychoactive substances. The screening analyses of blood and urine were performed by high performance liquid chromatography with diode array detection (HPLC-DAD), liquid chromatography with tandem mass spectrometry (LC-MS/MS) and (after derivatization) by gas chromatography-mass spectrometry (GC-MS). Synthetic cannabinoids are chemically very diverse and the isolation procedure must be versatile. Therefore, the synthetic cannabinoids were isolated from autopsy materials by precipitation with acetonitrile. It provided sufficient process efficiency while simplifying the procedure and significantly shortening the total time of sample preparation which is very important in methods used for routine laboratory work. The quantitative analyses were accomplished by LC-MS/MS method. Analyses were performed on an Agilent Technologies 1200 series liquid chromatograph connected to a 6460 Triple Quad mass spectrometer. Separation was achieved on a Kinetex C18 column (Phenomenex). The limit of detection of the quantitative method was 0.1 ng/mL for body fluids and 0.1 ng/g for solid tissues.

Results
AMB-FUBINACA and EMB-FUBINACA were detected and quantified in all post-mortem materials except the blood. The highest concentrations of AMB-FUBINACA and EMB-FUBINACA were revealed in the stomach content (5.8 and 36.2 ng/mL, respectively). The determined concentrations of these compounds in solid tissues were in the ranges 0.2–0.9 ng/g and 0.2-3.5 ng/g.

Discussion
Recent reports clearly show that the use of synthetic cannabinoids creates serious health problems. AMB-FUBINACA and EMB-FUBINACA are new synthetic cannabinoids whose pharmacological and toxicological effects are not well (or not at all) known. Recent studies indicate that the action of AMB-FUBINACA on the cannabinoid CB1 receptor is 85 times more powerful than THC, and 50 times more powerful than JWH-018. This synthetic cannabinoid is responsible for many (analytically confirmed) poisonings, including lethal ones. The massive outbreak of 33 cases of intoxication with this cannabinoid were reported in New York in 2016. In turn, many deaths related to AMB-FUBINACA were reported in New Zealand in 2017.

In the intoxication case discussed here, both AMB-FUBINACA and EMB-FUBINACA were not detected in the blood despite detection in the urine as well as other autopsy materials. The highest concentrations of the compounds were found in the stomach, and their concentrations in the intestine were also higher when compared to other materials. This may indicate oral administration of these synthetic cannabinoids. Such an assumption is also confirmed by significantly higher concentrations of AMB-FUBINACA and EMB-FUBINACA in the gastric contents when compared to stomach tissue. Oral administration of cannabinoids result in low blood concentrations due to the effect of the first pass through the liver, where they undergo extensive metabolism. This situation resulting in low blood concentrations is also characteristic for other cannabinoids, including THC after oral administration. As shown by the presented case, even in cases of fatalities, it is possible that the parent substance won't be present in the blood, while being present in other materials. Cases of deaths where synthetic cannabinoids were detected in internal organ tissues, but not in blood, were previously described in the literature.

In the discussed case, the medical examiner finally concluded that the man died due to acute respiratory failure and the most probable cause of death was the cardiotoxic action of AMB-FUBINACA and EMB-FUBINACA.

Conclusions
The case concerns the identification and quantitation of AMB-FUBINACA and EMB-FUBINACA in post-mortem human specimens. The presented case shows that synthetic cannabinoids can be undetected in the blood of even seriously or fatally intoxicated people. This situation means that the analysis of only blood samples may not confirm poisoning. Due to the extensive metabolism of the synthetic cannabinoids, the analysis of metabolites is one of the solutions to this problem. The presented case also suggests that AMB-FUBINACA and EMB-FUBINACA use is dangerous to health and may lead to fatal intoxication.
Abstract ID 48
Old drugs in modern times: Opium poisoning fatalities in Western countries.

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Aim
Opium is a substance extracted from Papaver somniferum L that is chronically abused specially in the traditional production regions. Reports on opium poisoning has largely disappeared from the occidental literature on drug problems but recent scientific articles show cases of death related to opium in industrialized countries. This work describes opium fatalities when the substance is used as a recreational drug to get euphoric effects including its abuse as a substitute of heroin in order to show circumstances and different patterns of consumption regarding availability and preparations.

Methods
Data were obtained by a computer assisted search of the literature in PubMed (National Center for Biotechnology Information, US National Library of Medicine, Bethesda, MD, USA) accompanied by a hand search of the reference lists of all articles for any relevant studies not included in the database. Search terms included "opium", "poppy", "Papaver somniferum" which were cross-referenced with outcome-based terms (and "poisoning", "toxicity", "overdose", "intoxication", "death", "hospitalization"). In addition, Google Scholar government reports on websites discussing data were reviewed, including the European Monitoring Centre from Drugs and Drug Addiction (EMCDDA), UN Office on Drugs and Crime (UNODC) reports, abstracts published by the Society of Forensic Toxicologists, the American Academy of Forensic Sciences, the European Association of Poison Centres and Clinical Toxicology, and the American College of Medical Toxicology were also reviewed. Articles were limited to the English, French, German and Spanish language. Documents or articles about "opium addiction" were excluded.

Results
We found seven studies of cases involving 15 fatally intoxicated subjects. In all of them toxicological analysis was documented. Our search encountered cases occurring from 1985 until 2018. All except two were male, the range age of the decedent was 17-42 years old. In terms of geographical origin, reported cases came from legal producers such as Hungary, Spain and France, and also in countries where poppies grow wildly such as Denmark. Four cases occurred in USA where the availability or accessibility to the poppies is not related to the previous legal or authorized circumstances. All of them except three had a history of alcoholism or/and drug abuse including abuse to heroin and cocaine. Route of administration and opium preparation: Injection: A brownish paste (containing morphine, codeine, papaverine) warmed up in a spoon over a candle and after evaporation of the moisture the remaining residue solved in water and poured onto a cigarette filter and aspirated into a syringe and injected (Hungary). Oral route: Opium was ingested as a homemade black paste called "rachacha" obtained by decocting poppy heads and evaporating water (France). We founded an overdose case due to ingestion of raw opium in a legal poppy field (Spain). In seven cases poppy capsules stolen from fields were used for the production of poppy tea (Denmark). Opium poppy seed pods were used to make tea or seeds shaken in water, poppy seeds bought at the grocery store (USA).

Discussion
Opium abuse in Western countries constitutes a good example of how old drugs comes to the arena in modern times. Poppy preparations have been used both in a non-dependent or dependent form and also to supplement opiates and to avoid withdrawal syndrome. Opium alkaloids are transferred onto the seed coats through the latex during the harvesting process and unprocessed seeds have high alkaloid content making them desirable for drug users. Both fresh and dried poppy capsules have been used for the production of tea and unwashed poppy seeds can be purchased online from both domestic and international sources with no current legal repercussions or regulations.

Conclusions
The appearance of cases of "rachacha" in France and Spain together with poisonings with opium tea have brought to the present a form of traditional consumption that returns to the forensic toxicology laboratories. The danger of this renewed interest in opium consumption is exemplified in that many of the poisoning cases reported resulted in fatalities. Folk knowledge is lacking and high doses are sought in order to obtain more effects than simple therapeutics. We would like to alert about this new trendy risk in Western countries, sometimes even encouraged by internet forums.
Abstract ID 50
Identification and quantification of NPS: U-47700, N-ethylhexedrone, adinazolam, 4-CIC and 4-CMC in evidence and biological material using LC-MS/MS-case report.

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Aim
We report the case of a 24 years old woman, found death in bed, at home. Because of her NPS addiction, she attended the drug rehabilitation. In the past she had suicide attempts. 5 ziplock bags with unidentified powders were found at the scene. Due to the suspicion that the death was caused by intoxication with the unknown substances, the autopsy and toxicological analyses of biological materials and powders located at the scene were performed. The autopsy showed no significant changes, but toxicological analyses indicated fatal intoxication with U-47700 taken in combination with others illicit drugs. There was no information about the route of administration of drugs.

Methods
Blood and urine samples (0.2 mL) were extracted with ethyl acetate from alkaline medium (pH 9). General screening and quantitative analyses were carried out using ultra-high performance liquid chromatography coupled with triple quadrupole tandem mass spectrometry (UHPLC-QqQ-MS/MS). Powders were dissolved in methanol, prior to general screening and quantifications also performed by UHPLC-QqQ-MS/MS. All methods applied were fully validated.

Results
In the presented case of fatal death the determined concentrations of drugs were as follows, in blood: U-47700 1474 ng/mL, N-ethylhexedrone 58 ng/mL, adinazolam 18 ng/mL, 4-CIC 8 ng/mL, 4-CMC 1.7 ng/mL, in urine: U-47700 3937 ng/mL, N-ethylhexedrone 147 ng/mL, adinazolam 82 ng/mL, 4-CIC 130 ng/mL, 4-CMC 417 ng/mL. Sertraline (blood - 89 ng/mL, urine - 32 ng/mL) was also determined in both materials. The same substances (separately) were found in 5 powders: U-47700 (12 % by weight), N-ethylhexedrone (54 %), adinazolam (14 %), 4-CIC (23 %), 4-CMC (26 %).

Discussion
Because of woman's NPS addiction, the concentration of illicit drugs in urine can permanent oscillate on some levels. Due to this fact, interpretation of toxicological results indicated lethal intoxication with opioid - U-47700, taken in combination with other substances: cathinones, benzodiazepine and SSRI. However, literature data indicate a strong synergistic interaction of U-47700 with benzodiazepines, to which adinazolam belongs. For this reason, combination of adinazolam and U-47700 could have also contributed to the death. It is also known that cathinones may react with SSRIs, resulting in occurrence of the serotonin syndrome, but this combination in presented case was not involved in the death.

Conclusions
To the best of our knowledge, we reported the first fatal case involving combination of U-47700, N-ethylhexedrone, adinazolam, 4-CIC, 4-CMC and sertraline. The developed high-sensitive and high-selective methods allow to determine those substances in biological material.
Abstract ID  52
Determination of boldenone in postmortem specimens including blood and urine samples using LC-MS/MS.
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Aim
Boldenone (BOLD), one of androgenic anabolic steroids (AAS), although banned in humans, is still available illegally. AAS abuse has previously been associated with various cardiovascular adverse events including acute myocardial infarction, arrhythmia, and sudden death. In this study, the concentration of BOLD was determined in postmortem specimens including peripheral and heart blood, muscular tissue, urine sample from the corpse of a human male who intentionally injected BOLD undecylenate into his shoulder muscle. In addition, the endogenous levels of BOLD in the blood and urine samples of young human males have been reported.

Methods
A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method with solid-phase extraction (SPE) was developed and validated for the analysis of BOLD in blood, muscular tissue and urine samples.

Results
The validation parameters including linearity, accuracy, precision, matrix effect, and recovery were satisfactory. The concentrations of BOLD in the blood of 20 young human males who didn't take BOLD were under the limit of quantitation (LOQ, 0.5 ng/mL). Additionally, the mean level of BOLD in the urine samples was 3.19 ± 1.65 ng/mL (range: 0.37–6.02 ng/mL). The concentrations of BOLD in the victim's blood from the femoral vein and heart were 140.44 and 25.74 ng/mL, respectively. On the other hand, those in the muscular tissue from the injection site and the urine sample were 142.3 ng/g and 3474 ng/mL, respectively.

Discussion
The level of BOLD in the blood from the femoral vein was similar to the muscular tissue and higher than that in the blood from the heart, suggesting that the drug was administered by an intramuscular injection. It can be explained due to slow hydrolysis of BOLD undecylenate and continuous release of BOLD to peripheral blood. The concentrations of BOLD in the blood and urine sample in this case were higher than the endogenous BOLD level.

Conclusions
A new analytical method with SPE and LC-MS/MS system was developed for the analysis of BOLD. This method was sensitive, selective and can be successfully applied for the detection and quantification of BOLD in blood, muscular tissue and urine samples. Since few studies have reported the fatal levels of BOLD in the blood and other postmortem biological specimens in humans, we had no reference therapeutic, toxic or fatal concentrations of BOLD. The medical examiner did not find any clear anatomical or pathological cause of death in this case but only estimated a possibility of sudden death caused by the administration of BOLD.
Abstract ID 78

Deaths linked to synthetic cannabinoid - CUMYL-PEGACLONE.

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Aim

CUMYL-PEGACLONE (CP) is a synthetic cannabinoid belonging to the family of CUMYL-derivatives and is considered a full CB1 and CB2 receptor agonist. The CUMYL-derivatives are recognised as a potent group of synthetic cannabinoids even though the toxicity of this particular substance and its contribution to death are relatively unknown. Since 2017, six deaths involving the presence of CP use have been identified in Victoria, Australia; these will be presented as part of a case summary.

Methods

In Victoria, Australia the role of the Coroner is to investigate unexpected deaths to determine the identity of the deceased, the cause of death and, in some situations, the circumstances surrounding the death. The Coroner will also seek to identify and make recommendations on how similar deaths may be prevented in the future. The population of Victoria is ~6M with ~39,000 deaths reported each year; of these ~6500 (17%) are referred to the coroner, of which ~5,800 cases receive toxicology analysis. Toxicological analysis consists of screening and quantification for alcohol (GC-FID) and a comprehensive range of prescription and illicit drugs (tandem LC/MS/MS) in post-mortem (preferably femoral) or ante-mortem blood and other associated postmortem specimens and/or exhibits.

In suspected drug deaths additional testing was conducted on case exhibits which led to subsequent targeted analysis for CP in blood. Confirmation of CP (m/z 374) was performed by LC/MS/MS using three product ions m/z 256, 91 and 167. Prior to these cases being tested, CP was not included as part of the standard toxicology investigation regime.

Results

The deaths consisted of five males and one female aged from late twenties to mid-forties all suspected of using drugs. In all cases synthetic cannabis material and/or associated drug paraphernalia was discovered at the scene. The analysis of exhibits revealed the presence of CP, 5F-CUMYL-P7AICA, MDMB-CHMICA and methylamphetamine. CP post-mortem femoral blood concentrations ranged from 1.0 to 8.6 ng/mL (median 2.9 ng/mL). A range of other drugs was also detected including THC, methylamphetamine, pregabalin, codeine, diazepam, temazepam, olanzapine, zuclopenthixol and alcohol.

Discussion

Four deaths were determined as drug-related. Interestingly, two individuals were found to have significant heart disease co-existent with their synthetic cannabis use. The cause of death was determined to be unascertained in the other two cases where the additional drugs detected were not deemed significant. In these cases the pathologist noted contribution to the cause of death by CP could not be excluded. Adverse and toxic effects of CP have not been previously reported. Transdermal absorption of other CUMYL-derivatives has been reported to cause dry mouth, nausea, vomiting, mydriasis, blurred vision, dizziness, balance disorder, ataxia, weakness, numbness, palpitations, tachycardia, orthostatic hypotension, somnolence, lethargy and confusion [1]. The stability of novel psychoactive substance (NPS) compounds is not well established and the detection of CP and other synthetic compounds may have been affected given the delay in testing (up to 3 months).


Conclusions

Further monitoring and assessment of CP in coronial cases is needed to determine the potential risk of this drug to public health and safety.

Keywords : coronial, synthetic cannabinoids, NPS, CUMYL-PEGACLONE, toxicology, death.
Abstract ID 81

Determination of 7 psychotropic drugs and metabolites in hair by LC-MS/MS: identification of quetiapine acute poisoning using hair root.

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Aim

The analysis of hair matrix has gained increasing importance in forensic toxicology, and is useful for therapeutic drug monitoring in hair sample. The study aims to develop an analytical procedure for the simultaneous determination of seven psychotropic drugs and metabolites (quetiapine, 7-hydroxyquetiapine, midazolam, α-hydroxymidazolam, clonazepam, 7-aminoclonazepam, clozapine, N-demethylclozapine, ketamine, norketamine, paliperidone, paroxetine) in hair using liquid chromatography combined with electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS). And the method has been successfully applied in the identification of quetiapine and its metabolite 7-hydroxyquetiapine in hair root after a single dose administration.

Methods

The sample preparation is simple with hair sample pulverized in methanol. The extract was separated by liquid chromatography using acetonitrile-acetate buffer (10mmol/L ammonium acetate and 0.1% formic acid) as mobile phase, and then analyzed by LC-MS-MS system in the multiple reaction monitoring (MRM) mode via positive electrospray ionization (ESI+).

Results

The limits of detection ranged from 0.002 to 0.05 ng/mg for all analytes. Good lineairities were observed for each analyte over the linear range, with correlation coefficients over 0.995. The intra-day and inter-day precisions (RSD) were less than 20%, and accuracies ranged from 85.3% to 112.9%. This method of detection was applied to the analysis of guinea pig hair roots after a single dose administration.

Discussion

Quetiapine and 7-hydroxyquetiapine were both detected in guinea pig hair roots from 5 min post administration. The concentration of quetiapine (10.3-1733.8 ng/mg) was much higher than that of 7-hydroxyquetiapine (0.1-40.6 ng/mg) in the hair roots of guinea pigs, and higher concentrations of quetiapine and 7-hydroxyquetiapine occurred in black hair than in white and brown hair.

Conclusions

The established method is simple, rapid and sensitive, and appropriate for identification and quantification of seven psychotropic drugs and metabolites in hair. The animal experiment demonstrated that hair roots may be a good specimen for proving acute quetiapine poisoning when other biological matrices are not available.

Abstract ID 85

Stability studies of cocaine compounds in biological fluids during post-analysis custody.

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Aim

To evaluate in vitro stability of cocaine compounds, cocaine (COC), benzoilecgonine (BE), ecgonine methyl ester (EME) and benzoilecgonine ethyl ester or cocaethylene (EBE), in blood and urine, during post-analysis custody. Parameters evaluated were: time of custody, temperature, addition of preservative (blood) and pH (urine).

Methods

Blood and urine samples were spiked with the four analytes to give a final concentration of 1000 ng/mL. The prepared samples were divided into 2 groups and stored at two temperatures (4 ºC and -20 ºC). Each one of these groups was subsequently divided in other two groups: with and without preservative (NaF) for blood, and pH 4 and 8 in the case of urine. COC, BE, EME and EBE were analyzed by GC-MS after SPE and derivatization with BSTFA. Analyses were performed in triplicate every two weeks for a year.

Results

In blood samples the influence of the temperature is of paramount importance. At -20ºC all the compounds have demonstrated to be stable, with recoveries, after one year of custody, higher than 80%, both in samples with and without preservative. On the contrary, degradation was observed in the four studied compounds when the samples were maintained at 4ºC. In these conditions, the influence of the preservative was also noticeable and a higher stability was found in samples added with NaF. COC and EBE had a similar profile and both compounds disappeared after 30 days in samples without NaF and after 150 days in samples added with NaF. EME disappeared after 185 days and after 215 days in samples with and without preservative, respectively. BE recoveries, after 365 days of custody, were 68.5% (in samples with NaF) and 3.7% (in samples without NaF).

In urine samples the four compounds were stable in all the studied conditions, with the exception of samples at pH 8 and stored at 4ºC. All the compounds disappeared in these conditions, COC and EBE after 75 days of custody and EME after 15 days. The exception was BE, with a recovery of 23% after one year of custody.

Discussion

Conclusions

The influence of storage temperature is much higher than urine pH or the influence of adding preservative in blood samples.

The best storage conditions for samples from cocaine consumers are in the freezer, at -20ºC. In addition, blood samples must be added with NaF and urine samples must be buffered at pH 4.
Abstract ID  91
Insufflation of Ethyl chloride: a case report
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Aim
Ethyl chloride or chloroethane is a flammable gas (b.p. 12°C) that has been used as a refrigerant, solvent and chemical intermediate. It is employed therapeutically as a topical anaesthetic spray for the control of pain in athletic injuries and muscle spasm. This compound has also become a popular substance of abuse as a street-drug that can be purchased at various retail shops. Its engagement as anxiolytic in recreative sniffing to “get high” is gaining popularity among youngsters. Inhalation of ethyl chloride vapor can result in dizziness, euphoria, confusion, incoordination, hallucinosis, impairment of short-term memory and narcosis also for brief exposures, if at high concentrations. Acute mortality generally does not occur at concentrations below 50,000 ppm. The pharmacokinetics of chloroethane in human has not been investigated, but some experimental studies on mice confirm that in mammalian tissues is oxidatively dechlorinated in an NADPH- and O2-dependent reaction, P450-dependent metabolism, resulting in the formation of acetaldehyde, or conjugated with glutathione (GSH) in a reaction catalysed by GSHS-transferases. Forensic cases reporting toxicological concentrations on tissues of this inhalant are scarce in literature i.e. to the best of our knowledge, very few old cases are present in literature, thus diagnosis of ethyl chloride intoxication may be difficult to assess with no reference levels available. We describe a case of fatal intoxication due to ethyl chloride sniffing. Medico-legal and toxicological investigations are presented and concentrations on different tissues are provided. Blood, vitreous humor, lung and brain samples were collected at the crime scene and during autopsy.

Methods
Ethyl chloride determination was performed by dynamic headspace gas chromatography coupled to mass spectrometry (GC-MS) by using an Agilent 7890A GC/5975C system (Santa Clara, USA) coupled to thermal desorption extractor ENCON Evolution (EST Analytical, Ohio, USA). Capillary column was an Agilent Technologies CP Wax (diameter: 0.25 mm, length: 60 m, and film thickness: 0.25 μm). Sample preparation consisted in the addition of sodium sulphate to biological material placed in a 20-mL headspace. Single ion monitoring (SIM) acquisition mode was used for ions m/z 64, 49, 28. Quantitation was by external standard calibration.

Blood alcohol determination was performed by head-space gas chromatography coupled to flame ionization detector (HS-GC-FID). Traditional drugs of abuse (cocaine, morphine, codeine, cannabinoids, amphetamines, methamphetamines, ketamine, methadone) were searched by GC-MS on blood after solid phase extraction and derivatization. Benzodiazepine and tricyclic antidepressant determination in blood were performed by LC-MS/MS.

Results
A 40-year-old white male (height 178 cm, weight 71 kg, body mass index 22.4) was found dead in his bedroom. The entire flat was in order, with no signs of violence and all the premises closed. Leftovers from a meal were found in the kitchen. No suicide notes were found. The corpse held a rag loosely on his mouth. Four cans (2 empty, 2 partially empty) containing EC and labelled as cryo-anaesthetic were found next to the body. Two more empty cans containing ethyl chloride were found in the trash basket in the bedroom. The cans and the rag were collected from the scene and a fragment from the rag was cut and stored in a sealed 20 mL head-space vial. Ethyl chloride was detected in all analysed samples. Peripheral blood concentrations (0.156 g/L) were slightly different from central blood concentration (0.203 g/L) collected during autopsy, however the difference was not significant. Lung and brain samples showed ethyl chloride concentration of 30 and 45 mg/kg, respectively. Ethyl chloride present at a much lower concentration in vitreous humour compared to blood (0.027 vs 0.203 g/L). Important differences in ethyl chloride concentrations between vitreous humour and other tissues were also observed in the previous findings of Broussard et al. (2000) which found 0.012 vs 0.423 g/L, for vitreous humour and blood, respectively. This could be related to an unfavourable partition equilibrium in this district or to physical barriers that prevent gas diffusion.

The search for other toxicants or volatiles in blood was negative, except for small quantities of ethanol (0.1 g/L), which hardly could contribute to the cause of death.

Discussion
Unfortunately, literature is scarce about cases of ethyl chloride toxic exposure and only few cases may be helpful in the interpretation of toxicological results. As for procedural aspect, loss of ethyl chloride was prevented by using tight-seal head-space vials, in fact no important differences were observed between results of blood collected at crime scene (0.165 g/L) and at the autopsy (0.156 g/L). In case of inhalant intoxication, it is of utmost importance to safely collect and store the material sampled both during crime scene investigation and autopsy.

Conclusions
As no other drug related or alternative causes of death was found, death was subsequently attributed to an overdose or adverse reaction to ethyl chloride.
Abstract ID  120
Determination of drugs in histological material by liquid chromatography–tandem mass spectrometry.
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Aim
In the course of retrospective forensic-toxicological investigations, body fluids and tissue samples may be unavailable. Thus, alternative matrices, such as histological samples, have to be necessarily analyzed. Here, we present a procedure to determine drugs in histological material and the results obtained by the qualitative analysis. This targeted analysis was based on the information from the case files and further sources.

Methods
The histological material (liver, kidney, heart, brain, lung) was cut out of paraffin block and roughly chopped with a scalpel. Next, a cryo mortar was used to crush the material. Internal standard mixture was added to a defined and weighed quantity of crushed material. After an extraction with methanol, a solid-phase extraction with a Biotage HCX-column was performed. The resulting extract was evaporated and reconstituted in mobile phase at starting conditions. Analysis was performed by high performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) using positive electrospray ionization and multiple reaction monitoring mode. In addition to analyzing the real samples, a mixture of the relevant analytes was spiked to blank histological material to prove the effectiveness of the used method.

Results
Different basic drugs (ajmaline, diazepam, doxepin, fentanyl, loperamide, metoprolol, midazolam, verapamil) were successfully proven in histological material by the applied method. Thereby, these were not necessarily detectable in all investigated materials. In addition, several drugs were not detectable in the investigated histological material of the presented cases. Apart from the histological samples, postmortem liver, kidney and heart blood was available in one of the cases. Ajmaline was proven in these postmortem samples as well as in the histological liver and heart samples. Contradictory, ajmaline was not detectable in the histological kidney sample. In accordance with the results of the postmortem heart blood sample, fentanyl and midazolam were proven in the histological heart sample.

Discussion
Analyzing various tissue samples was decisive for the successful proof of most of the drugs. Depending on the examined analyte, one to three cases were investigated. Thus, a pattern for the detectability of the above mentioned drugs depending on the analyzed histological tissue sample is not derivable. In our study, analytical results in postmortem matrices were not necessarily compatible with findings in the corresponding histological samples.

Conclusions
The applied procedure can be used to determine basic drugs in histological material. A successful determination of these drugs may depend, for example, on the variety of histological tissue sample as well as their content in the slice and their ability to remain in the tissue during histological procedure.
Abstract ID 127
Method for the quantification of heroin biomarkers and their metabolites using LC-MS-MS in postmortem specimens.

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Aim
Although blood is the specimens of choice for drug related fatalities investigations, other types of samples have benefits and, as complementary specimens, can provide a higher degree of confidence for toxicologists in their final reports. Furthermore, blood may not be available in some cases; due to bleeding, burns, decomposition and/or urination before death. The most practical approach in such cases is to use alternative post-mortem specimens for analysis, but these specimens are not well studied making it harder for toxicologists to interpret their results. The aim of this study was to report the blood (B), vitreous humor (VH), urine (U), liver (L), kidney (K), bile (B) and stomach contents (SC) of heroin metabolites in deaths involving heroin using fully validated LC-MS-MS method for confirmation of 6-monoacetylmorphine (6-MAM), morphine (MOR), 6-acetylcodine (6-AC) and codeine (COD) and obtained new information regarding to the interpretation of origin, time and source of morphine in the tested specimens.

Methods
An LC-ESI-MS-MS method was developed and validated for the determination of 6-Monoacetylmorphine (6-MAM), 6-Acetylcodine (AC), Morphine (MOR), and/or codeine in unhydrolysed post-mortem specimens. Twenty post-mortem cases positive for opioid use were investigated, all cases were male with exception of one, the mean age of the deceased was 37 (median 30, range 21 to 70 years old).

Results
Twenty positive post-mortem cases were involved in this study. The mean blood MOR levels were in the range of 0.004 to 1.200 mg/L with an average and median concentrations of 0.162 and 0.099 mg/L, respectively; MOR in stomach contents, U, VH, bile, liver and kidney were in the range of 0.03-0.79 mg/L, 0.86-4.114 mg/L, 0.026-0.109 mg/L, 0.040-3.870, 0.015-0.81mg/kg and 0.026-0.109 mg/L, respectively The mean blood MOR levels were in the range of 0.004-1.222 mg/L with average and median concentrations of 0.222 and 0.98 mg/L, respectively. MOR concentrations in stomach contents, U, VH, bile, liver and kidney were in the range of 0.034-1.515 mg/L, 0.045-42.264 mg/L, 0.005-0.487 mg/L, 0.030-9.808 mg/L, 0.025-2.095 mg/kg and 0.095-0.505 mg/kg with averages (median) concentrations of 0.19 (0.12) mg/L, 1.198 (0.614) mg/L, 0.060 (0.045) mg/L, 1.130 (0.491) mg/L, 0.300 (0.113) mg/kg and 0.0468 (0.245) mg/kg, respectively. The heroin marker (6-MAM, codeine and 6-AC) was detected in all stomach contents specimens. Heroin biomarkers were not detected in liver samples and only one case was positive for 6-MAM in kidney specimens. Free morphine ratios were calculated for VH/B, Liver/B, KID/B, Bile/B and U/B and the mean ratios of free morphine were 0.05-4.19, 0.2-3.88, 0.25-5.94, 0.15-176.93 and 0.38-153.93 folds with average (median) ratios of 0.8 (0.33), 0.4 (0.16), 2.8 (2.59), 33.80 (11.55) and 21.01 (4.65) folds.

Discussion
Although the weather in Saudi Arabia is very hot most of the year making the 6-MAM hydrolysis to morphine more likely to happen, the addition of sodium fluoride as a preservative prevented 6-MAM from converting to morphine in most of the cases. Additionally, the availability of urine, vitreous humor, and stomach content samples provided valuable information to which opioid(s) has been administrated. In the current work; heroin metabolites have been detected in biological fluid and tissue i.e. liver, kidney, gastric content, bile, liver, and stomach contents; new information on the distribution of these analytes were reported; AC a heroin biomarker, for example, was detected in stomach contents for the first time in the this work.

Conclusions
This report highlights the importance of multiple specimen testing in heroin related deaths. This provides new information on the distribution of opioid analytes; for example, this is the first time AC, a heroin biomarker, has been detected in stomach content and vitreous humor samples.
Abstract ID 135
Integrating toxicology into the diagnosis of sudden cardiac death.
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Aim
The use of drugs (licit and illicit) and toxic substances, such as ethanol, can increase the risk of sudden death (SD) in young apparently healthy adults. The objectives of this study were: 1) to investigate the impact of the toxicological results found in cases of sudden death and 2) to relate the toxicological results with the clinical characteristics, pathological and genetic findings of the cases.

Methods
Consecutive SD in people aged between 16 and 50 years was included over a 3-year period. All medico-legal autopsies SD with toxicology studies were included. Comparison between toxicological data and demographic characteristics, clinical circumstances, pathological and genetic results were also included. Positive toxicology was defined as the presence of any substance (licit and/or illicit). Causes of SD were classified as toxic, cardiac (SCD), sudden unexplained death (SUD), respiratory, neurological and others. The variants identified in the genetic study have been classified using a specific standard terminology: pathogenic (P), likely pathogenic (LP), variant of uncertain significance (VUS), likely benign (LB), or benign (B).

Results
101 cases were included. They were predominately male (84%) with a middle age (39.8 ±8.6 years). 1) 52 (51.5%) cases had positive toxicological findings. Toxic compounds were considered the first cause or a fundamental cause of death in 25 (24.8%) cases. Ethanol was the most frequently identified agent (69%), following by licit drugs (56%) and drugs of abuse (39%). Cocaine (n=13), diazepam (n=11) and olanzapine (n=4) were the most commonly found drugs. Illicit drugs were at toxic levels (85.7%, p<0.001) while psychotropic drugs were at non-toxic levels (82.6%, p<0.001). 2) Cases with positive toxicology were younger (≤ 30 years) than negative cases (37.9±9.1 vs. 41.9±7.8; p=0.02). Patients with more than 3 comorbidities showed an association with positive toxicological results (n=14 vs. n=3; p=0.017). Polypharmacy was detected in 12% (n=6) of the cases. SCD occurred in 49 cases, SUD in 17 cases and death from respiratory causes in 6 cases. The toxicological results were mostly negative in the presence of SCD (p<0.001) and in SD cases with respiratory causes (p<0.05). In SUD, toxicology results were mainly positive, although the difference between positive and negative groups was not statistically significant (p=0.084). Genetic study was performed in 69 (68.3%) of SD cases. P or LP variants were identified in 12 cases. These variants were more frequent in cases with negative toxicology, 8 (25.8%) cases, than in cases with positive toxicology, 4 (10.5%) cases. VUS were reported in 29 cases (42.0%) and LB or B in 2 cases (2.9%). Specifically, genetic testing was performed in 16 of the 17 SUD cases. Only in 2 (12.5%) of these cases, P or LP variants were identified (1 case with positive toxicology and 8 (50%) cases presented VUS (6 cases with positive toxicology).

Discussion
Systematic toxicological investigation in cases of SD is always necessary. Results obtained should be interpreted along with the rest of the studies carried out. Toxicology results contribute to the implementation of targeted interventions aimed at improving healthy lifestyle and therapeutic measures to avoid future episodes based on individual and family risk profiles.

Conclusions
Multidisciplinary cooperation to reach the diagnosis of deceased cases is necessary as well as in the cases of clinical strategies to avoid deaths in at-risk patients.
Abstract ID 146
Death from diabetic ketoacidosis in the Eastern part of Denmark in 2016-2018
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Aim
The aim of this study was to evaluate cases with diabetic ketoacidosis (DKA) as a cause of death in a three-year period from 2016 to 2018. We were especially interested in studying DKA deaths without a history of diabetes mellitus. In addition, it was evaluated whether acetone could replace betahydroxy butyrate (BHB) as an indicator of diabetes as acetone is easily analyzed together with ethanol on headspace gaschromatography.

Methods
All autopsy cases (1394) investigated at the section of Forensic Chemistry were screened for ketoacidosis by quantification of BHB and acetone in blood or muscle and glucose in vitreous humour and/or urine. Cases with known or suspected diabetes mellitus or BHB ≥ 1000 µM were also analysed for HbA1C.

BHB levels < 300 µmol/L were considered as normal. BHB levels above 500 to 1,000 µmol/L as raised. BHB levels above 1000 µmol/L were defined as high levels, and blood levels > 2,000 µmol/L as indicative of ketoacidosis.

Acetone levels above 0.01 g/L were considered as high levels. Blood concentrations were used for comparison of BHB and acetone (1187 autopsy cases).

The DKA cases were extracted from our LIMS system (laboratory information management system, StarLims Abbott Informatics) by searching for cases with either a high BHB and/or a high HbA1C. The autopsy reports were reviewed to select cases diagnosed DKA and to elucidate known and unknown history of diabetes.

Results
Thirty two cases (2.3%) were concluded to be DKA-related deaths. Eleven (34%) of these were undiagnosed cases, where all were men. Age range were 31-63. Median age: 45. Men dominated (67%) the diagnosed cases. Age range of the diagnosed cases were 29-80, Median age: 54.

Correlating BHB and acetone in blood demonstrated that all cases with blood BHB above 2000 µmol/L would be included with an acetone cut off set to 0.01 g/L. Several cases (N=54 ~4.5%) with raised blood BHB from 500 to 1000 µmol/L and some cases (N=15~1.3%) with high blood level BHB from 1000 to 2000 µmol/L was not detected. Moreover, few cases (12~1.0%) had blood BHB values below 300 µmol/L, while acetone was above 0.01 g/L.

Discussion
DKA seems to be more widespread among middle-aged men either because they don’t respond to symptoms of diabetes or none-willingness to follow a treatment. Although, this study presents a limited data set on DKA related deaths, it is in agreement with findings elsewhere [1]. Ali et al [1] found that about a third of all DKA deaths in a 6 year period were unknown diabetes. This underlines the importance of having tools to discover these cases. It is important to measure vitreous glucose and HbA1C along with blood BHB/acetone to distinguish DKA from other types of ketoacidosis.

Conclusions
In the light of the high number of undiagnosed diabetes cases, we recommend to measure vitreous/urine glucose and HbA1C in cases with elevated BHB (≥ 1000 µM). Screening for acetone with a cut off 0.01 g/L would include all DKA cases (BHB > 2000 µM). A minor number (1.3%) with high level BHB was not detected. Therefore, it can be concluded that acetone is sufficient for screening for high ketone levels and thereby a diabetic disorder.

Abstract ID 155

A Death Involving Barbital – An Old Psychoactive Substance (OPS).

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Aim

The aim of this presentation is to remind toxicologists that drugs that may no longer be in use, in this case barbital, can still contribute to death. Although history and investigation can be critical in determining appropriate testing when performing a general unknown analysis, this case also serves as a reminder that unconfirmed immunoassay results should not be immediately dismissed as false positives.

Methods

This case was submitted to NMS laboratories as a routine comprehensive postmortem toxicology examination. Cardiac blood was screened for volatiles by dual-column GC-headspace-FID analysis, for barbiturates, cannabinoids and salicylates by ELISA (reporting limits of 40, 10 and 120 ng/mL, respectively) and for over 250 targeted compounds using LC/TOF-MS.

Results

Initially, reported confirmed findings were 120 mg/dL ethanol (GC-headspace FID) and 33 ng/mL alprazolam by LC-MS/MS. Barbiturates were positive by ELISA but did not confirm by GC/MS with selected ion monitoring for amobarbital, butabarbital, butalbital, pentobarbital, phenobarbital and secobarbital at a reporting limit of 200 ng/mL. At the time laboratory results were reported no case history had been provided. Later discussion with the forensic pathologist revealed that barbital was a suspected agent in this case. Directed testing for barbital by GC-FID was performed based on this new information. A 6-point calibration curve (0.50-50 mcg/mL) was utilized to determine the barbital concentration in this case which was initially over range and ultimately reported as 380 mcg/mL after appropriate dilution of the sample.

Discussion

Case investigation revealed the decedent, a 31 year old white male, was found by his parents in his bedroom laying face up on the ground at the foot of his bed wearing a t-shirt and shorts. On the top of his dresser was an unknown white powder in close proximity to a spoon which appeared to contain a similar white substance. A small garbage can in the bedroom also had a zip lock sandwich bag containing a white powdery substance in it. A backpack at the scene contained a brown colored laboratory bottle containing a white powder labeled Barbital Sodium Merck (1/4 lb). A prescription vial labeled sertraline, 50 mg was also in the bag with no tablets remaining, consistent with the date filled on the prescription. The backpack also contained a silver scale in a black leather case. Empty alcohol containers were found under the bed and in a desk drawer. According to his ex-wife, the decedent was reportedly in a Ph.D. program in Chemistry and had recently dropped out and he had had a history of addiction (alcohol and cocaine). There was a history of depression and mention of suicide to relatives. Autopsy findings were unremarkable except for a fatty liver consistent with chronic alcoholism and pulmonary congestion and edema. No needle tracks or hesitation marks were found.

Barbital (Veronal®) is one of the first barbiturates used medicinally and dates back to 1903. Barbital is a long acting barbiturate with a half-life of approximately 48 hours. While it is no longer used as a sedative hypnotic in the USA, it is frequently employed in chemistry laboratories as a buffer solution. In this case it was sodium barbital that was found at the scene. The age of the bottle could not be determined but was clearly vintage and could have been on the shelf of the chemistry laboratory in which the decedent had worked based on the inventory number written on the bottle. As barbital is no longer used medicinally, it is often not in the confirmation tests employed by toxicology laboratories. In this case, while the unconfirmed positive ELISA screen would suggest the presence of a barbiturate not included in the confirmation panel, it was the case history and the analysis of the powder at the scene by a crime laboratory that resulted in additional testing being ordered which confirmed the presence of barbital in blood. Barbital concentrations in three other cases reported in the literature where death was due to this drug ranged from 312-579 mcg/mL consistent with the findings in this case.

Conclusions

Based on the scene findings and the toxicology results, the cause of death was determined to be due to a combined drug (barbital, ethanol and alprazolam) toxicity. The absence of a suicide note and the history of drug and alcohol abuse resulted in the manner of death being determined as accidental. This case serves as a reminder that old psychoactive substances may still be involved in deaths, even when they are no longer in common use.
Abstract ID 182

Simultaneous determination of fentanyl, its metabolite norfentanyl, acetylfentanyl, butyrfentanyl, furanylfentanyl and octafenial in whole blood with GC-MS.

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Aim

Fentanyl analogues have entered the illegal drug market during the last years and have been related to many overdoses and deaths worldwide. Acetylfentanyl, butyrfentanyl, furanylfentanyl and octafenial are among the most common of them. These fentanyl analogues are very potent substances that show opioid-like effects and can be lethal in micrograms. Therefore, sensitive and specific methods are necessary to be developed for the determination of these substances in post-mortem biological fluids along with fentanyl and its main metabolite norfentanyl.

Methods

A gas chromatography-mass spectrometric method was developed and fully validated for the simultaneous determination of fentanyl, norfentanyl, acetylfentanyl, butyrfentanyl, furanylfentanyl and octafenial in whole blood. The developed method includes protein precipitation with acetonitrile and solid-phase extraction using mixed-mode Bond Elut Certify columns followed by derivatization with TFAA. Analysis was carried out using a DB-5MS fused silica column at selected ion monitoring (SIM) mode. Methadone-d3 was used as internal standard. The mass fragments used for identification of the analytes were: m/z 245, 189 and 202 for fentanyl, m/z 150, 229 and 132 for norfentanyl-TFA, m/z 231, 146 and 188 for acetylfentanyl, m/z 259, 146 and 189 for butyrfentanyl, m/z 283, 240 and 158 for furanylfentanyl, and m/z 279, 176 and 236 for octafenial. The mass fragments for methadone-d3 were m/z 287, 72 and 161. The developed method was fully validated according to international guidelines by using the following criteria: selectivity, specificity, sensitivity, linearity, absolute recovery, precision and accuracy.

Results

The LOD and LOQ of the developed method were 0.15 and 0.50 ng/mL, respectively, for fentanyl, norfentanyl, acetylfentanyl and butyrfentanyl, whereas the respective values for furanylfentanyl and octafenial were 0.30 and 1.0 ng/mL. The calibration curves were linear (R2≥0.990) from 0.50 to 50.0 ng/mL for acetylfentanyl and butyrfentanyl, and from 1.00 to 100.0 ng/mL for furanylfentanyl and octafenial. Absolute recovery was higher than 85% for fentanyl, its metabolite and its analogues. The accuracy (%Er) was found to range between -9.1 and 7.2%, whereas the precision (% RSD) was less than 8.3% for all analytes. The validated method was applied in 70 blood samples from positive cases for classical drugs of abuse (opiates, cocaine, cannabis, amphetamines) and no fentanyl analogues were detected. Fentanyl and nor fentanyl were detected in only three cases.

Discussion

There are growing concerns worldwide about the misuse and abuse of fentanyl and its analogues. The small therapeutic range of fentanyl makes its use, in the form of patches, for both medical and non-medical purposes possibly harmful. Both physicians and pharmacists should be aware of the relative risks and advise patients with chronic pain that are using fentanyl patches, to monitor frequently their blood fentanyl levels in order to prevent toxic effects. Opiate addicts that occasionally abuse fentanyl or fentanyl analogues should be aware of the high potency of these substances and the possible lethal synergy with other CNS depressants that can be developed. Furthermore, coroners and pathologists should be alert about the high toxicity of fentanyl analogues. Screening for fentanyl and its analogues should always be performed during the investigation of forensic cases concerning drug addicts.

Conclusions

The developed method will be useful for the investigation of clinical and forensic cases where fentanyl or the studied fentanyl analogues are involved. The preliminary results from the application of our method for the toxicological investigation of forensic cases of the Department of Forensic Medicine and Toxicology of the National and Kapodistrian University of Athens show that the prevalence of these fentanyl analogues in Greece is still limited.
Abstract ID 188
MDMA Intoxication in Potential donor with cardiac arrest –
A case report.
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Aim
Amphetamines consumption is still an important public health issue, namely in terms of compounds variability and disposition to consumers. However, some of them, still classic substances, keep standing in the illicit market, with remarkable and continuous success. That is the case of MDMA. Nevertheless, there is always new information and data on MDMA intoxication, both in vivo as in post-mortem context. The authors report an intoxication case with MDMA in an 18 years old male, who was considered a potential organ donor, after a cardiac arrest.

Methods
Whole blood samples were collected in vivo, at the Emergency Room (ER), in different moments, and post-mortem, at the Forensic Pathology Service of the National Institute of Legal Medicine and Forensic Science.

After a general screening procedure, samples were extracted by an SPE procedure (OASIS® MCX) followed by a GC-MS single quad analysis.

Results
The post-mortem whole blood sample was positive for Lidocaine (< 500 ng/mL), compatible with ER intervention, and positive for MDMA (2278 ng/mL) and MDA (49 ng/mL), while the whole blood samples collected in vivo (while the individual was maintained under advanced life support), were positive for MDMA (between 504 ng/mL and 1918 ng/mL) and MDA (between 20 ng/mL and 89 ng/mL). The samples were negative for other substances, namely ethanol, other drugs of abuse and medicines.

Discussion
Interpretation of these results is pivotal to understand the behaviour of the substance. So, in this case, MDMA post-mortem behaviour should be carefully evaluated, considering as possible influencers, in the specific context of the case, the time lapse between the death verification, the maintenance of the advanced life support and the body manipulation for organ collection purposes. Also referred and discussed is the ante-mortem/post-mortem ratio of MDMA obtained values, compared with literature references.

Conclusions
No doubt that death was due to MDMA intoxication, but information from the in vivo samples analysis suggests that this type of sample should also be considered, in a complementary role, whenever possible.
New Psychoactive Substances (NPS)-Related Deaths in Taiwan During the 2012–2018 Period.

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Aim

According to UNODC World Drug Report, illicit markets of new psychoactive substances (NPS) have been growing worldwide. NPS represent significant analytical and interpretive challenges to forensic toxicologists. With no or little knowledge of their pharmacological properties, these substances pose serious harms to the society. This study reports the analytical methodologies, toxicological findings, and trends of the 349 known NPS-related fatalities in Taiwan during the 2012–2018 period.

Methods

Postmortem blood samples (including femoral and cardiac blood samples) were routinely screened using liquid-liquid extraction (Toxi-tubes® A), followed by GC/MS, LC/ion-trap/MS, and LC-QTOF/MS analysis with automated library search protocol developed earlier in this laboratory. LC separation was achieved using an Agilent Zorbax SB-Aq (2.1 mm × 100 mm, 1.8 µm particle) analytical column operated at 50 °C. The mobile phase consisted of 0.1% formic acid (v/v) in water and methanol at a flow rate of 0.32 mL/min. NPS were quantified by LC-MS/MS using deuterated analog internal standards and fully validated protocols. Limits of quantitation for these NPS ranged from 0.5 to 10 ng/mL.

Results

Among the 349 postmortem cases included in this study, the mean age of the NPS-related fatalities was 28.8, ranging from 15 to 54, while 268 (77%) of these deaths were men and 81 (23%) were women. Top ten NPS detected in fatal cases and their mean concentration were: ketamine (n = 250; 22%, 1.2 µg/mL), PMA (n = 172; 15%, 2.8 µg/mL), PMMA (n = 107; 9.3%, 1.9 µg/mL), nimetazepam (n = 84; 7.3%, 0.15 µg/mL), ethylone (n = 80; 7.0%, 2.2 µg/mL), methylone (n = 71; 6.2%, 4.1 µg/mL), mephedrone (n = 70; 6.1%, 3.7 µg/mL), N-ethylpentylone (n = 69; 6.0%, 0.90 µg/mL), 5-MeO-MiPT (n = 53; 4.6%, 0.063 µg/mL), and 4-chloroamphetamine (n = 42; 3.7%, 0.58 µg/mL).

Discussion

Ketamine abuse has been a serious problem in Taiwan, but the percentage of its detection has decreased from 41% in 2012 to 20% in 2018. Nimetazepam was first detected in 2017; it then became the most frequently detected drug in 2018 (21%). Four synthetic cathinones were amongst the top ten NPS detected in postmortem cases. The prevalence of ethylone and methylone were decreasing, while mephedrone and N-ethylpentylone increased significantly. There was evidence of poly-drug use in 95% of these NPS-positive cases, including 1 to 13 other drugs. Average number of NPS detected per case increased from 1.9 in 2012 to 4.6 in 2018. Furthermore, NPS were ruled as the cause of death in 259 cases (74%), with falling as the second (n = 40, 11%) cause of death in these NPS-related cases.

Conclusions

NPS were commonly found in postmortem specimens in Taiwan, indicating these substances' global endemic nature. Over the last few years, the number and type of NPS have been increasing as the market continues to be changing. Laboratory findings indicated a dramatic increase in NPS-related fatalities. Synthetic cathinones and ketamine play significant roles in fatalities in Taiwan. With no or limited understanding on these substances’ toxicity, addiction potential, and withdrawal symptoms, growing use of NPS is certainly a critical public health issue. Effective detection and identification of NPS in the laboratory facilitates the collection of data and is helpful to the formation of supply reduction and health intervention strategies.
Abstract ID 206

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Aim
Glyphosate and glufosinate are non-selective, broad spectrum and highly polar herbicides, which are often used in poisoning and suicide cases. Due to their high polarity, the detection of these pesticides is difficult. The aim of this work is to develop a simple and rapid ion chromatography−tandem mass spectrometry method (IC-MS/MS) for the analysis of glyphosate and glufosinate in human blood without the need of derivatization.

Methods
The blood samples were precipitated with acetonitrile, and then the supernatant were purified by a Dionex OnGuard II RP column and a Dionex OnGuard II Ag/H column, respectively. The final extract was diluted 5-fold with deionized water in a plastic 2 mL vial ready for IC-MS/MS detection. A IonPac AS-11 column with KOH solution as eluent was used for chromatographic separation, and multiple-reaction monitoring (MRM) acquisition was conducted in the ESI negative mode for MS/MS analysis. This IC-MS/MS method was successfully applied to the analysis of several real blood samples obtained from postmortem examination in suicide cases.

Results
The limits of detection (LOD) and quantitation (LOQ) of glyphosate in blood were 0.02 μg/mL and 0.05μg/mL, respectively, with a good linearity range of 0.05-10 μg/mL, while the LOD and LOQ of glufosinate were 0.1 μg/mL and 0.3 μg/mL with a linearity range of 0.3-100 μg/mL. In one case, a 47-year-old woman committed suicide by overdose of glyphosate, the concentration of glyphosate in her blood was 4.9μg/mL; while in another case, the concentration of glufosinate in the blood obtained from a suicide was 2.7μg/mL.

Discussion
The sample preparation of the biological samples is vital, especially for the IC-MS/MS analysis, because the mobile phase of IC system doesn’t have organic solvents, the matrix in samples may remain on the column without an appropriate sample pretreatment. In this work, a Dionex OnGuard II RP column and a Dionex OnGuard II Ag/H column were used to clean the samples, which can eliminate the organic matrix and abundant Cl- in the blood samples, respectively. Furthermore, an organic solvent from an additional pump was added, after the conductivity detector of IC and before the MS, to aid the desolvation within the ion source. The results showed that the signal intensity didn’t change significantly.

Conclusions
This method was demonstrated to be simple, rapid and reproducible, and has broad application prospects in the analysis of glyphosate and glufosinate in toxicology.

Abstract ID 207
Determination of the dimethyl sulfide in the hydrogen sulfide poisoned blood by headspace gas chromatograph mass spectrometry analysis(HS-GC/MS).

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Aim
Being a stable metabolite of hydrogen sulfide, dimethyl sulfide has been utilized as an index for hydrogen sulfide poisoning. The aim of this work is to develop a simple headspace gas chromatograph mass spectrometry analysis(HS-GC/MS) method for the analysis of dimethyl sulfide in human blood and to apply this method in reality.

Methods
Forensic sample collection was performed as a routine part of our forensic work, following the autopsy guidelines and ethical guidelines of the Chinese Society of Legal Medicine. Deionized water(0.5mL) and phosphoric acid(85%,0.5mL) were added to the poisoned blood(1mL) . The dimethyl sulfide in poisoned blood was evaporated by microwave heating(Lv.3,30s) and separated by chromatographic column of GS-GasPro (30 mx0.32mm, 0.25 µm) , then determined by full scan or selective ion monitoring (SIM) mode of MS.

Results
The limits of detection(LOD) and quantification(LOQ) of dimethyl sulfide in blood were 0.02μg/mL and 0.05μg/mL. A good linearity was obtained at the concentration range of 0.1-10 μg/mL in blood sample, with the correlation coefficient of 0.999. The relative standard deviation was 3.42%-5.85% and the average rates of recovery was 92.8%-95.6%. This method was successfully applied to the analysis of several real blood samples obtained from postmortem examination in real cases.

Discussion
Although quantitative data are lacking, toxicity studies suggest that H2S gas is absorbed rapidly through the lungs in humans, it is then distributed to the blood. Researchers have identified a mammalian thiol S-methyltransferase which catalyzed the sequential methylation of H2S to methanethiol (CH3SH) and dimethyl sulfide(CH3SC3H). The methylation reactions were regarded as a detoxification mechanism since the mono- and dimethylated products had been described as progressively less toxic than H2S. The determination of hydrogen sulfide in blood can be used as direct evidence for the confirmation of poisoning. But it has always been a difficult point in the field of forensic toxicology. Acute poisoning occurs quickly and the content in the body is low. Therefore, how to accurately determine the concentration of dimethyl sulfide in the hydrogen sulfide poisoned blood is particularly important.

Conclusions
The established method was demonstrated to be sensitive, simple, specific and accurate. It can be applied in the detection of dimethyl sulfide in toxicology.
Abstract ID 210
Determination of Bromadiolone, Brodifacoum and Emamectin Benzoate in Human Whole Blood by LC-MS/MS.

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Aim
The samples in this study came from a real case during our routine work. The suspect involved in this case confessed that bromadiolone, brodifacoum and emamectin benzoate were used for committing crime. A LC-MS/MS method for determination of bromadiolone, brodifacoum and emamectin benzoate in human whole blood by liquid chromatography-mass spectrometry (LC-MS/MS) were established.

Methods
the effects of different mobile phase and purification on the recovery was studied. Human whole blood samples were extracted by acetonitrile. The supernatant was filtered with a 0.22 μm organic membrane, and then determined by LC-MS/MS. Gradient elution was performed on an Agilent Eclipse Plus C18 column(2.1 mm ×100 mm, 1.8μm). The mobile phases consisted of 0.1% formic acid aqueous solution and acetonitrile. The flow rate was 0.4 mL/min and the column oven was maintained at 40°C. The electrospray ionization source was used, and the positive and negative ion switching mode was used to detect it. The samples were detected by multiple reaction monitoring (MRM) modes.

Results
Spiked bloods were used for establishing determination method. The method aiming at the detection of bromadiolone, brodifacoum and emamectin benzoate is linear from 10 to 300 ng/mL, and the linear coefficients were 0.993, 0.991 and 0.989. Precision and accuracy were acceptable at any quality control level and the average recoveries were 72.5%, 71.3% and 67.7%, respectively. The method is very sensitive, and limits of detection (LOD S/N=3) of bromadiolone, brodifacoum and emamectin Benzoate were all 0.1ng/mL. The lowest limit of quantification (LOQ S/N=10) was 1 ng/mL.

Discussion
GC-MS and LC-MS are two kinds of common methods in toxicology analysis. However, bromadiolone, brodifacoum and emamectin benzoate could not be determined by GC-MS because of their high molecular weight. The detection of emamectin benzoate was performed by tandem mass spectrometry with electrospray ionization inpositive mode (ESI +) and the detection of bromadiolone, brodifacoum was in negative mode (ESI -). The retention times for emamectin benzoate were 1.99 min. The retention times for bromadiolone and brodifacoum were 2.31 min and 2.73 min respectively. Multiple reaction monitoring (MRM) using the precursor to product ion of m/z 886.5→126.0, m/z 527.0→522.8→134.9 were performed to quantify emamectin benzoate, bromadiolone and brodifacoum, respectively. The method is suitable for the identification of cases involving emamectin benzoate, bromadiolone and brodifacoum.

Conclusions
The method is very fast, easy to perform, and high sensitivity, which could be used for identification and quantification of bromadiolone, brodifacoum and emamectin benzoate in human whole blood from living people and dead bodies. Using this method, bromadiolone, brodifacoum and emamectin benzoate in blood of real case were all determined simultaneously.
Abstract ID 213
Suicides by inhalation of inert gasses: toxicological issues in 5 cases.

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Aim
Asphyxia represents an extremely widespread choice in the implementation of suicide. Asphyxiations can be categorized into three types: suffocation, neck compression, and involvement of chemicals or gasses. In this latter category, the used chemical or gase prevents the oxygen from either being transported in the blood or being used by the cells. Here we report 5 suicidal cases where the following gasses were involved: nitrogen, helium and argon.

Methods
All bodies underwent a complete autopsy and the following specimens were taken: central and peripheral blood, brain, lungs, liver, kidney, adipose tissue and ilopsoas muscle. A comprehensive systematic toxicological analysis was performed on post-mortem samples to investigate the presence of alcohol and volatile substances, drugs of abuse and prescription drugs.

A general screening for drugs of abuse and prescription drugs was performed in peripheral blood by liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) as described elsewhere.

For alcohol and volatile substances (nitrogen, helium, argon and other gases) a qualitative and quantitative analysis was performed by head space gas chromatography – mass spectrometry (HS-GC-MS). Briefly, 0.5 mL of blood was diluted 1:1 with dionized water, added with 0.5 internal standard (ter-butanol) and then was placed into the vial for the analysis. In addition, 1g tissues were weighed and diluted 1:4 with dionized water and then homogenized using a Dremel 985370 homogenizer (BioSpec Products, Inc., Bartlesville, OK, USA) and placed into sealed vials. A 1 ml of gas volume was injected onto the GC system. The headspace analysis was performed on a Shimadzu 2010 gas chromatograph with an FID detector, equipped with a Rtx®-BAC2 column measuring 30 m × 0.53 mm ID × 2.0 μm (Restek Corp., Bellefonte, PA, USA).

Results
Toxicological data obtained from the analysis of post-mortem biological samples detected in case 1 the presence of nitrogen (blood 86%, brain 67%, lung 80%, liver 75%, kidney 74%, adipose tissue 81%, muscle tissue 76%), in cases 2,3 and 4 the presence of helium (blood, range 0.2-1.15%; brain, range 0.12-0.31%; lung, range 0.47-2.30%; liver, range <LOQ-0.13%; kidney, range <LOQ-0.03%; adipose tissue, range <LOQ-0.11 %; muscle tissue, range 0.01-0.45%), in case 5 the presence of argon (blood 7.9%; brain 3.5%; lung 15.8%; liver, kidney and adipose tissue <LOQ; muscle tissue 7.2%). Neither alcohol nor other drugs were detected in all cases.

Discussion
In the five cases here reported the main mechanism of action of these gasses has involved the “displacement” of oxygen at the level of the pulmonary alveoli, thus eliminating the hypercapnic stimulus. The detection of inert gasses, by virtue of their chemical and physical characteristics and their extreme volatility, presents multiple technical problems in the performance of autopsy and toxicological investigation making it necessary to take special precautions for adequate sampling and for a correct analysis. In addition, a number of factors may later influence the results as, e.g. a longer period of time between death and sampling or pre-analytical artefacts during sampling of such highly volatile substances. Therefore, based on the cases here analyzed and the review of the most recent literature about this topic, it is recommended to properly sample more biological matrices, taking into consideration the analytical challenges required by the gaseous state of these substances.

Conclusions
To increase the reliability of chemical-toxicological analysis for inert gasses it is important to minimize the time of exposure of samples to atmosphere; their storage (fluids and tissues) must therefore take place in containers with sealed closure. In case of suspected inert gas intoxication, it is also necessary to pay attention to the method used for the detection of the analyte: normal carrier gas will not be able to highlight the presence of inert compounds and therefore it will be necessary to change carrier or, depending on the compound under investigation, the detection method. In the five cases here reported, the autopsy was unremarkable and only circumstantial data and toxicological investigation has allowed to correctly identify the cause of death.
Abstract ID 218
Fatal intoxication by intravenously administrated extract of Ricinus communis seeds.

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Aim
The castor plant (Ricinus communis) is a perennial plant from the family Euphorbiaceae. Its seeds are widely used as a source of castor oil. The seeds contain a lectine protein ricin, which is a water-soluble and highly toxic compound. Among others, castor plant contains an alkaloid ricin, which serves as a marker of ricin intoxication. Castor plant is considered as one of the most toxic plants. Several authors present lethal intoxication caused by castor beans ingestion. The aim of this work is to report a fatal intoxication by ricin together with determination of the poisoning biomarker ricin in various matrices.

Methods
A 25-year-old man in suicidal intent administered intravenously an aqueous extract of 13 castor seeds. After approximately 1 hour after the administration, he sought medical help. He was hospitalized for four days and then released for home care. Next day he was found at home with a consciousness disorder, transported to the local hospital where he died after another 10 hours.

External examination of the deceased man revealed injection punctures in different locations. Internal examination showed signs of lungs edema. Under the visceral pleura, there were numerous ecchymoses. The liver was steatotic in section. The stomach contained charcoal; small and large intestine were empty. The brain showed signs of severe edema, the gyri were flattened, and the sulci were narrowed. At the base of the brain, there were deep pressure coni. All organs were congested and large blood vessels and the heart contained liquid blood.

Toxicological examination of the biological material was focused on identification and determination of ricin concentration in clinical samples (blood and urine) as well as in the samples collected at autopsy (femoral blood, urine, and vitreous humor). The material was extracted by a simple liquid-liquid extraction. The analysis was conducted by a liquid chromatography hyphenated with a q-trap tandem mass spectrometry (LC-MS/MS, Q-trap 4500, AB Sciei). The limit of detection (LOD) and the limit of quantification (LOQ) of the method is 0.05 ng/ml and 0.2 ng/ml, respectively.

Results
The obtained results shown that ricin was present in the clinical samples (5 days after the intoxication) at levels of 3.5 ng/mL for blood and 3.3 ng/mL for urine, respectively. Furthermore, ricin was also found in the post-mortem samples in concentrations from 0.95 to 1.15 ng/ml in blood obtained from different locations (e.g. lower limb, upper limb, etc.). Ricin concentrations were determined in the liver, kidney and spleen in levels of 0.78, 0.86 and 0.83 ng/g, respectively. The determined levels of ricin reveal that its distribution is homogenous resulting in a weak redistribution phenomenon only.

Discussion

Conclusions
Findings in this case lead to a conclusion that the LC-MS/MS method is sufficiently sensitive for the ricin detection and determination. Ricin can effectively serve as an intoxication biomarker, which can be found in the body several days after poisoning.
Abstract ID 221
Distribution of antidepressant drugs in vitreous humor
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Aim
Antidepressants are widely used to treat depression and other psychiatric disorders. The antidepressant drugs mainly used in Greece are amitriptyline, nortriptyline, citalopram, clomipramine, fluoxetine, maprotiline, mirtazapine, sertraline and venlafaxine. The use of alternative biological materials in Forensic Toxicology has been highlighted by many researchers especially in cases of lack of classical biological samples such as blood, urine, etc. or in cases of putrefaction. Vitreous humor is an alternative biological sample that can be used for the analysis of the antidepressant drugs due to its analytical advantages compared to other alternative biological samples.

The aim of this work was to study the distribution of antidepressant drugs in vitreous humor through the determination of concentration ratios of the antidepressant drugs in blood to the respective in vitreous humor. For this purpose the development and validation of a GC/MS method for the simultaneous determination of antidepressant drugs and their metabolites as well as its application during the investigation of real forensic cases was necessary.

Methods
An analytical method was developed for the simultaneous determination of nine antidepressant drugs (amitriptyline, nortriptyline, citalopram, clomipramine, fluoxetine, maprotiline, mirtazapine, sertraline and venlafaxine) and four of their metabolites (desmethylsertraline, desmethylvenlafaxine, desmethylmaprotyline and desmethylmirtazapine) in vitreous humor with the technique of gas chromatography in combination with mass spectrometry. The developed method includes solid-phase extraction using mixed-mode non polar cation exchange Bond Elut Certify columns followed by derivatization with Heptafluorobutyric Anhydride (HFBA) at 70 °C for 30 min. Protriptyline was used as internal standard. Analysis was carried out using a DB-5MS fused silica column at selected ion monitoring (SIM) mode. After proper validation the developed method was applied in 24 vitreous humor samples from different forensic cases where antidepressants were involved. The respective blood samples were analyzed using an in-house developed and validated method.

Results
The developed method presents significant advantages over already published methods for the determination of antidepressant drugs in vitreous humor using GC/MS as it allows the simultaneous determination of 9 antidepressant drugs and 4 of their metabolites. For all analytes, LOD and LOQ were 1.50 and 5.00 ng/mL, respectively, and the calibration curves were linear within the dynamic range of 5.00-500.0 ng/mL (R2≥0.990). The absolute recovery was found to be ≥86.3% for all analytes. The accuracy (%Er) was found to range between -6.58 and 6.18%, whereas the precision (%RSD) was less than 10.9% for all analytes. The developed method was successfully applied to vitreous humor samples from 24 blood positive cases for antidepressant drugs. These samples were collected within 2 days after death. The vitreous humor concentrations were compared to the concentrations of the respective post-mortem blood samples. The blood/vitreous humor concentration ratios were also calculated and ranged from 0.26 to 12.64. Citalopram, mirtazapine and its metabolite desmethylmirtazapine as well as venlafaxine and its metabolite desmethylvenlafaxine were the most identified substances in these samples (n≥4) and were more thoroughly studied. For citalopram the blood to vitreous humor ratio was found between 1 and 1.73, for mirtazapine 1.34-1.99, for desmethylmirtazapine 1.22-2.12, for venlafaxine 1.0-1.49 and for desmethylvenlafaxine 1.11-1.70.

Discussion
Our results are consistent with the limited work of other studies and suggest that vitreous humor could be an appropriate matrix for the screening of antidepressants in postmortem toxicology. Vitreous humor was proved to be a significant biological sample for the qualitative analysis of antidepressants as in all cases where an antidepressant drug was detected in blood samples, the respective antidepressant drug was also found in vitreous humor samples but with a significantly wide range in the concentration ratios. Due to this it is difficult to estimate the blood concentrations from vitreous humor concentrations in cases where blood samples are not available. When vitreous humor concentrations are higher than those of blood concentrations, probably the intake of antidepressant drug took place a lot of time before death, while in cases where blood concentrations are higher the drug intake was close to death time.

Conclusions
Our results confirm that vitreous humor can be a useful alternative biological specimen in forensic toxicology for the determination of antidepressant drugs especially when blood and/or urine are not available. The study will be continued with more samples in order the blood/vitreous humor ratios to be more accurately determined.
Profile of poisoning cases in JSS Medical College & Hospital - A two year prospective study.

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Aim
1. To determine the socio-demographic profile of medico-legal cases reported to JSS Medical College & Hospital
2. To determine the mortality rate
3. To determine the profile of different medico-legal cases in poisoning

Methods
This is a prospective study which was conducted in the casualty of JSS medical college & Hospital from October 2016 to September 2018 and the main source of this study was medico-legal register which comprises of information regarding various parameters.

Inclusion criteria: All cases brought to the casualty department with known history of medico-legal implications related to poisoning

Exclusion criteria:
1. cases with inadequate details/ missing data.
2. Cases referred from other hospitals & clinics

Results
A total of 2033 medico-legal cases were registered during the time period of October 2016 to September 2018. Among these, 374 poisoning cases were reported. Within this 215 were male and 159 were female. Majority, 295 of the poisoning cases were for suicide followed by 75 cases were due to accidental poisoning and 4 were homicidal in nature. The following were the common poisoning agents detected among patients who succumb with history of consumption poison, total deaths occurred were 30.

1. In 2016, 06 cases were due to dimethoate, paraquat 01, phosphide 06 and carbamate 01.
2. In 2017, 03 cases were due to dimethoate, paraquat 02, phosphide 01 and carbamate nil.
3. In 2018, paraquat 01, phosphide 08 and carbamate 01.

Discussion
The results were analysed and compared with the other studies conducted at various places. In the present study, male preponderance was observed and out of total 374 cases 295 (14.5%) cases were suicide, 4 (0.2%) were homicide and 75 (3.7%) were accidental in nature and overall 18.4% of the medico-legal cases were due to poisoning. On the basis of demography, 71.7% were from the urban background and 28.3% were from rural area. This finding was consistent with studies done by SN Hussaini et al, chandrappa Siddappa and Bharath Kumar.

Conclusions
The present study shows that 18.4% of medicolegal cases attending JSS Hospital were due to poisoning. Major cause of poisoning was suicidal in nature and Organophosphorus compounds were major products used for poisoning. Public health authorities should develop and implement preventive strategies with comprehensive approach to reduce the incidence of mortality.
Abstract ID 225
Ethanol production by S. aureus under controlled experimental conditions: evaluation of applicability in postmortem cases.
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Aim
The aim of this contribution was to study the microbial ethanol production by Staphylococcus aureus, a Gram-positive, aerobic/ facultative anaerobic bacterium, under controlled experimental conditions and model the correlation between the microbial produced ethanol and the other alcohols.

Methods
S. aureus was cultured at 25°C, under strict anaerobic conditions in Brain Heart Infusion (BHI) culture medium, for 30 days. In a parallel experiment, S. aureus was cultured aerobically for the first 5 days and anaerobically until the 30th day, at 25°C in the same culture medium. Ethanol and the other alcohols concentrations were determined by head space-gas chromatography-flame ionization detector (HS-GC-FID) in 24 h intervals for both sets of experiments. Acetonitrile was used as internal standard. Regression analysis was employed to model the correlation between the microbial produced ethanol (as the response variable) and the other higher alcohols (as the explanatory variables). All linear models were produced by Ridge Regression (RR), a L2 regularization technique specifically targeting data that suffer from intercorrelated independent variables. In RR a penalty is applied to the sum of the squared coefficients in the optimization function, pulling big coefficients towards zero. Hence, RR decreases model variance and avoids overfitting. The applicability of the models was tested in postmortem cases.

Results
1. S. aureus produced higher concentrations of ethanol, 1-propanol and methyl alcohol under anaerobic conditions compared to the respective levels under mixed aerobic/ anaerobic conditions. The concentrations of isobutanol and 1-butanol were comparable for both sets of experiments. In constructing the mathematical models for predicting the produced ethanol, isobutanol, 1-butanol, methyl-butanol, and 1-propanol were the most significant for S. aureus under mixed aerobic / anaerobic conditons, while isobutanol, methyl-butanol, 1-propanol and then 1-butanol were most significant for S. aureus under strict anaerobic conditions. The linear model constructed under mixed conditions had a better fit (R² 0.678) compared to the one under anaerobic conditions (R² 0.420) indicating probably that the anaerobic model does not follow a linear trend.

Discussion
In previous research we showed that the amount of ethanol produced by microorganisms, such as Escherichia coli, Clostridium perfrigens and Clostridium sporogenes, depends on the co-produced higher alcohols (1-propanol, isobutanol and methyl-butanol) and 1-butanol. The correlation between the levels of the alcohols and the concentration of the microbial ethanol was expressed by mathematical models. The applicability of these models in real postmortem cases is beneficial, where the origin of postmortem ethanol needs to be clarified.

Conclusions
Our results show that the mathematical modeling of the ethanol production under controlled experimental conditions for S. aureus is feasible, as a first approximation to the quantification of the microbial ethanol production in cases where other alcohols were produced simultaneously with ethanol, as it has been proposed by our research group for other microbial species.
Abstract ID 227
Identification and quantification of antipsychotic in blood samples by LC-MS/MS: cases reports and data of 3 years of routine analysis.
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Aim
Antipsychotic drugs (APs) are widely prescribed for the treatment of schizophrenia and psychosis. The pharmacological treatment of schizophrenia is often performed with the simultaneous use of two or more antipsychotic agents to achieve the desired control of psychotic symptoms. Available APs include both conventional (typical) and new (atypical) antipsychotic medications. Atypical APs, such as quetiapine, now account for the vast majority of AP prescriptions. Quetiapine is an effective agent for the short- and long-term treatment of bipolar disorder. However, an increased risk of mortality in addition to cardiovascular complications have been reported in patients suffering from dementia when treated with atypical APs. In forensic toxicology, APs are of considerable interest because of their potential abuse and their involvement in intoxications and suicides.

A quantitative liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay was developed for the simultaneous determination of 16 APs (amisulpride, aripiprazole, chlorpromazine, clorazepine, cyamemazine, fluphenazine, haloperidol, levomepromazine, melperone, olanzapine, paliperidone, prometazine, quetiapine, risperidone, sulpiride, ziprasidone) in blood samples of postmortem cases.

Methods
Chromatographic analysis was preceded by an optimized solid-phase extraction procedure using Oasis® HLB (3 cc, 60 mg) extraction columns (strongly hydrophilic sorbents, reversed-phase) and 0.5 mL of blood samples. Qualitative analysis was performed in central blood samples and peripheral blood was selected for the corresponding quantitative analysis. The quality control and the calibration samples were prepared by spiking drug-free blood samples with methanolic working standard solutions. Clomipramine-d₃, olanzapine-d₈ and zolpidem-d₆ were used as internal standards. The extracted analytes were separated by UPLC (Waters) with a reversed-phase Acquity UPLC® HSS T3 (2.1x100 mm id, 1.8µm) column in a gradient mode (0.1% formic acid and acetonitrile) at a 0.5 mL/min flow rate and a chromatographic run-time of 8 min. The Waters TQD triple quadrupole LC/MS system was operated under the multiple-reaction monitoring mode (MRM) using an electrospray ionization (ESI) interface.

Results
For the detection and quantification of APs in blood samples, the authors developed and validated a LC-MS/MS method according to international accepted criteria and guidelines. The method was selective and linear in concentration range of 5-500 ng/mL (r²>0.995). The lower limits of quantifications (LLOQs) corresponded to the lowest concentrations used for the calibration curves. Both within and between-day coefficients of variation were below 10%. No significant interfering compounds, matrix effect or carryover were observed for these drugs. Olanzapine must be analyzed quickly because this analyte degrades quite rapidly after extraction. The method was successfully applied to authentic forensic samples.

The authors retrospectively examined APs positive cases detected in samples collected during autopsies performed in the Forensic Clinical and Pathology Service of National Institute of Legal Medicine and Forensic Sciences centre branch or in other autopsies carried out in the central region of Portugal.

Between January 2016 and December 2018, the Laboratory of Forensic Chemistry and Toxicology received 3588 requests for toxicological analysis: 1413 cases were positive for drugs from which 329 (25%) cases were positive for APs, 58% from male individuals and 42% from female. Quetiapine was the most prevalent APs (32%) followed by olanzapine (18%). During this period, there was 25 postmortem cases with APs blood concentrations above therapeutic range, in which 88% of those are in agreement with the information received (psychological history or acute intoxication suspicion) and the manner of death was suicide.

Discussion

Conclusions
The LC-MS/MS method showed to be appropriate for identification and quantification of APs in whole blood samples. It has proven to be selective, linear, accurate and precise for all studied drugs. The retrospective study indicates that antipsychotics is an increasingly prevalent class of drugs. Antipsychotic drugs must be measured not only in toxic concentrations but also in therapeutic levels in postmortem cases therefore, the importance of a sensitive method to cover the low therapeutic range in which APs are usually presente.
Abstract ID 228

Wonkyung Yang
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Aim
Methamphetamine is a highly addictive central nervous system stimulant and one of the most abused drugs in South Korea. It causes multiple social problems related to crime, traffic and non-traffic accidents, physical and psychological hazards. In recent years, there have been large increases in production, distribution and the degree of methamphetamine abuse worldwide. This study assessed the trends in methamphetamine-related fatalities between 2005 and 2018 in the Seoul metropolitan area, South Korea.

Methods
Analytical results obtained from autopsy samples were investigated by the laboratory information management system of National Forensic Service (NFS). Bloods, hairs and tissues of the deceased in central area of Korea were taken from autopsy cases by NFS for 14 years (2005 - 2018). Gas chromatography/mass spectrometry (GC/MS) and Liquid chromatography/tandem mass spectrometry (LC/MS/MS) were used for the detection of methamphetamine and other drugs. The counts of cases, age, gender, the causes of death were investigated in methamphetamine-related postmortem cases for this period.

Results
There were 112 mortality cases related to methamphetamine use over a 14 year period and 71.4 % of those cases happened between 2014 and 2018. The mean age of victims was 42.3 years and 75.0 % of those were male. Deaths were due to drug poisoning (33 cases), natural disease (27 cases), suicide (31 cases), accident (12 cases) and homicide (7 cases). The blood concentrations of methamphetamine in poisoning deaths were 0.60 – 47.10mg/L, which were higher than those in other causes of death (0.07 – 17.53 mg/L). Most common methods of suicide were hanging and falling due to psychopathological problems. Most natural diseases related to cases were cardiovascular diseases and haemorrhagic strokes.

Discussion

Conclusions
Methamphetamine-related deaths have been increased in the Seoul metropolitan area, South Korea, from 2014 to 2018 compared to previous years. The empirical results of this study highlight the risk of methamphetamine abuse in South Korea.
Abstract ID 230
Carbon Monoxide Poisoning: comparison of spectrophotometric and gas chromatographic methods for quantification under controlled storage conditions.

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Aim
Misdiagnoses of carbon monoxide (CO) poisonings leading to severe long-term neurological sequelae and death have been reported increasingly in recent years. One of the main causes are the discrepancies in results obtained from methods used for quantification, which include spectrophotometric and gas chromatographic approaches. In post-mortem cases, analyses are often carried out with a time-delay, ranging from hours to days or weeks. Thus, it is important to implement adequate sample collection and storage procedures, which need to be specific for the matrix and analyte of interest. Given the lack of updated studies on the topic for CO poisonings, we aim to evaluate the effects of storage conditions on the quantification in blood of carboxyhemoglobin (COHb) and total blood CO (TBCO), in order to improve the methods for CO quantification and provide not only the optimal storage conditions, but also estimate expected losses in non-optimal circumstances and consequently generate a model to predict COHb and TBCO concentrations taking into consideration all storage parameters.

Methods
COHb and TBCO levels are monitored in CO-fortified blood samples over a period of one month, with analyses performed on days 0, 1, 2, 4, 7, 14, 21 and 28. Parameters evaluated include preservatives ethylenediaminetetraacetic acid (EDTA), sodium fluoride (NaF), lithium heparin (LiH) and sodium citrate (NaCit), temperatures of -20°C, +4°C and room temperature (RT), blood tube headspace (HS) volumes of <25%, 25-50% and >50%, initial COHb saturation levels with ranges of 10-20%, 30-40% and 50-70% as well as reopening and freezing/thawing-cycles. All blood samples are analysed by CO-oximetry, airtight gas syringe-gas chromatography-mass spectrometry (AGS-GC-MS) and samples of the tube HS are analysed by AGS-GC-MS. Results are evaluated and compared statistically with the software R.

Results
Linear regression of results obtained for COHb and TBCO show a moderately positive correlation (R² = 0.52). Through an additive step model selection process and analysis of variances (ANOVA) to determine the significance of effects of the storage conditions on changes in COHb and TBCO, a model without the blood tube HS was established to be the most suited for the dataset. A multiway-ANOVA with parameters time, saturation level, preservative and temperature showed significance of all conditions. A multiple linear regression (MLR) with the same parameters was performed for COHb and TBCO, showing statistical significance for all conditions except temperatures -20°C and +4°C, and the first four days of analysis for COHb. For TBCO, all conditions are significant except time. Comparison of reopened vs not reopened and frozen vs not frozen samples showed no statistical significance (p-values >0.05). A prediction model for COHb and TBCO based on the MLR with the investigated storage conditions was generated and tested on a second dataset. Comparison between measured and predicted concentrations showed no statistically significant difference (p-value >0.05) and strong positive correlation for both COHb (R²=0.99) and TBCO (R²=0.86).

Discussion
The moderate positive correlation between COHb and TBCO is not sufficient to explain the measurement results, raising doubts over previous studies using linear relationships between spectrophotometric and gas chromatographic COHb/CO measurements. TBCO shows higher concentrations, taking into account CO dissolved in blood but not detected as COHb, and thus may give more accurate amounts of CO and respective CO poisoning diagnosis. The amount of CO dissociating into the HS during storage and released during reopening of the tubes is found not relevant (dissociation rate < 0.001 %), therefore it was excluded from following evaluations. Similarly, freeze- and thaw-cycles do not alter COHb or TBCO concentrations sufficiently to require a restriction, thus rendering reanalysis of frozen samples possible. Derived from the MLR, the best storage conditions are to store the blood sample in an EDTA tube, with a HS volume of <50% and at least refrigerated. For COHb, concentration stability can be guaranteed for up to 4-7 days without significant changes, though small changes are observed even after one month of storage, if stored appropriately. For TBCO measurements, no significant alterations occur over the whole observation period, making it a more reliable biomarker for CO poisoning determination.

Conclusions
Controlled sampling and storage conditions are crucial for CO poisoning determinations, since concentration changes occur based on different conditions and time after sampling in different manners. TBCO seems to undergo the smallest variations over time for a period of one month and generally results in higher concentrations, making it a more accurate and reliable biomarker compared to COHb. Additionally, it has the potential to be employed in forensic cases, since it is not as subject to optical changes of blood as COHb measured by CO-oximetry. Nevertheless, studies into the correlation of TBCO levels with the severity of poisoning need to be carried out to allow better understanding of the results obtained. For the time being, a concomitant use of COHb and TBCO as biomarkers especially for challenging cases is encouraged, respectively under controlled storage conditions following our recommendations.
Abstract ID 234
Identification of unique markers of 4-methylmethcathinone (4-MMC) degradation in putrefied biological matrices.
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Aim
Drug degradation as a consequence of putrefactive bacterial activity is a well-known factor that affects the identification and quantitation of certain substances of forensic interest. Understanding the biotransformation of drugs by putrefying microorganisms is therefore important for drug detection in a decomposed biological matrix. Current knowledge on putrefaction-mediated degradation of drugs is, however, significantly lacking. It has been previously shown that 4-methylmethcathinone (4-MMC or mephedrone) is unstable and tends to degrade in post-mortem matrices. This study aimed 1) to investigate the degradation of 4-MMC by various putrefactive bacteria; 2) to elucidate the possible structures of the formed degradation products; and 3) to detect the degradation products in 4-MMC containing biological matrices following putrefactive processes. Collectively, the study was designed to assess if putrefactive degradation products of 4-MMC are suitable for drug monitoring purposes in post-mortem toxicology.

Methods
Four putrefactive bacteria were used in this study: Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae and Proteus vulgaris. Bacteria were grown in Oxoid CM1135 brain heart infusion broth. After adding 4-MMC, each culture was incubated at 37°C for 24, 48 or 72 h. 4-MMC degradation was also investigated in post-mortem human blood and fresh porcine liver macerate. These samples were left to putrefy in sample tubes at room temperature for one week with occasional opening of the tube caps to facilitate oxygen exposure. The samples (bacteria culture media, blood, liver macerates) were extracted with diethyl ether or ethyl acetate under alkaline conditions. The extracts were analyzed on an Agilent Technologies 1290 Infinity liquid chromatography (LC) system coupled to either an Agilent 6490 triple quadrupole mass spectrometer or an Agilent 6510 accurate mass quadrupole time-of-flight mass spectrometer. LC was conducted on a Poroshell 120 EC-C18 column. Electrospray ionization was performed in positive ionization mode. Structural elucidation was based on modern spectroscopic analyses including the use of high resolution mass spectrometry (HRMS) and nuclear magnetic resonance (NMR) spectroscopy.

Results
All four putrefactive bacteria were capable of degrading 4-MMC extensively under the experimental conditions explored. Many degradation products observed were considered to be specific to the putrefactive microorganisms used. Of particular interest was the discovery of a novel degradation product common to all four bacterial species. The product was assigned as 2-hydroxy-(4-methylphenyl)propan-1-one (HMP) based on the spectroscopic data. This degradation product was detectable in both post-mortem human blood and porcine liver samples spiked with 4-MMC following one week of decomposition.

Discussion
Putrefaction is the decomposition of the body’s soft tissues by micro-organisms, including bacteria, fungi and other non-fungal eukaryotes. Although the process itself is quite well documented, the effects of the putrefaction process on many drugs are still unknown. This knowledge gap creates many complications when interpreting toxicological results, especially for post-mortem cases where there has been a large degree of putrefaction. This study investigated the effect of 4 representative putrefying bacteria on 4-MMC. The study showed that the degradation products of 4-MMC observed were due to biotransformation action of bacteria and not due to other chemical means. Although putrefactive fungi were not specifically investigated, they may be present and contribute to the degradation of 4-MMC in porcine liver and post-mortem blood specimens under the putrefaction conditions used in this study. The stability of the identified degradation products especially HMP should be further investigated to assess their validity of serving as marker analytes for monitoring 4-MMC in post-mortem toxicology.

Conclusions
4-MMC is susceptible to degradation by putrefactive bacteria. Some of the identified degradation products, such as HMP, may serve as useful markers of 4-MMC in decomposed post-mortem specimens.
Abstract ID 237
Can UV-Vis spectroscopy be used to determine the % carboxyhaemoglobin in degraded blood and tissue samples?

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Aim
To develop a simple sample preparation technique to enable the measurement of % carboxyhaemoglobin (% COHb) in solidified blood and body tissue samples highly degraded by fire.

Methods
For each solid blood and body tissue sample, between 0.5 - 2g was placed into a glass vial and up to 3 mL of deionised water was added to achieve sufficient liquid sample volume for duplicate carboxyhaemoglobin analysis. Each sample was vortex mixed, sonicated for 10-20 minutes and centrifuged or up to 3 minutes to remove undissolved particulates. For analysis, up to 250 µL of liquid sample was added, based on sample colour and analyst experience, to a test tube containing 10 mL sodium dithionite (Sigma-Aldrich, UK) dissolved in 0.01 mol/L Tris buffer (Sigma-Aldrich, UK) and gently inverted. The absorbance of each sample was measured for the presence of carboxyhaemoglobin at 419.5 nm twice, once before and once after being saturated with carbon monoxide (BOC, UK) for 2 minutes. Saturation with carbon monoxide was performed in an externally vented safety cabinet under strict procedures and in the presence of a CO alarm. The ratio between the unsaturated and saturated absorbance was used to calculate the % COHb.

%COHb = Unsaturated absorbance (419.5 nm) x 100
Saturated absorbance (419.5 nm)

Absorbance was measured using a Perkin Elmer Lambda 35 UV-Vis spectrophotometer. Quality control (QC) samples prepared in blood at 3%, 25% and 65% COHb (Werfen, UK) were analysed with each batch to assess assay performance. Samples and QC's were analysed in duplicate for each batch.

Results
Highly degraded exhibits (n=18), comprised of 16 bloods and 2 tissues containing no liquid sample were analysed between July 2017 and December 2018. The liquid obtained from sample preparation ranged from dark red that mimicked blood to an almost colourless liquid. Acceptable unsaturated and saturated peaks were obtained in 6 of the 18 (33%) samples so that an approximate % COHb measurement could be reported, ranging from 39 – 78% COHb (mean 59.8%, n=6). COHb was reported as present in a further nine (50%) cases but no measurement was assigned due to poorer peak shapes observed during analysis. The remaining three (17%) samples, including the muscle and lung, either produced a liquid very pale in colour or contained too many interfering particulates to produce acceptable absorbance at 419.5 nm and were deemed too degraded for successful analysis.

Discussion
Measurement of carboxyhaemoglobin in blood is commonly requested in the event of a fire to help establish whether carbon monoxide poisoning could have contributed to the cause of death. The current methodology utilised by the Forensic Toxicology Department (Eurofins Forensic Services, UK) to measure % COHb requires blood samples to be in liquid form, making the analysis of degraded dried blood and body tissue samples caused by fire unachievable without sample preparation.

This method attempted to use deionised water to rehydrate samples to produce a liquid containing sufficient carboxyhaemoglobin to absorb at 419.5 nm without producing interfering particulates that would make it unsuitable for UV-Vis analysis. Where possible, blood was sampled from the middle of the samples in an attempt to retrieve the least degraded and most moist part of the sample. This was not possible for certain exhibits where all the sample was consumed. It was noted throughout analysis that samples containing a small amount of moisture provided the best results, whereas completely dry samples were generally unsuccessful.

Approximate measurements were obtained for six (33%) samples analysed with a mean % COHb of 59.8%, a level consistent with those reported in fatal cases. Carboxyhaemoglobin was deemed present in a further nine (50%) samples but no approximate measurement could be assigned due to the quality of the peak shapes. Three samples did not provide acceptable absorbance either before or after saturation with carbon monoxide and would suggest an insufficient amount of carboxyhaemoglobin within the sample. The tissues were among the samples that did not produce results, which was expected given the likely reduced amount of blood containing carboxyhaemoglobin remaining in these samples.

Due to their highly degraded nature, each sample was repeated at least twice using freshly prepared aliquots. This method uses an unsaturated and saturated reading from each sample to calculate % COHb and is not reliant on a calibration curve, therefore the sample volume, sonication and centrifuging parameters could be slightly altered within and between the samples without affecting the results. QC's prepared in whole blood were used to assess assay performance as it was not possible to mimic the nature of the casework samples within the lab.

Conclusions
A simple sample preparation technique can be used to provide approximate % COHb measurements in solid blood highly degraded by fire which may help establish cause of death in certain cases. The success of the technique is limited to samples with a certain degree of preservation allowing for the release of carboxyhaemoglobin for analysis.
Abstract ID 240
Have point-of-care devices a role in forensic toxicology?

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Aim
Laboratory methods currently used to measure glucose and ketones (acetone, acetoacetate and β-hydroxybutyrate (BHB)) can be expensive, time consuming and require considerable sample volume. Occasionally, limited volumes of samples are available at autopsy for biochemical and/or toxicological analysis. Indeed, though vitreous humour (VH) has been shown to be a suitable matrix for the assessment of glucose and ketones, it is often retrieved in limited volume. Appropriately governed glucose and ketone point-of-care (POC) devices have provided a convenient role in clinical practice for analyte testing near the patient utilising only small (5-10 µL) volumes of sample. In this study, we considered the potential application of the Abbott FreeStyle Precision Pro Blood Glucose/β-Ketone Meter POC device for post-mortem (PM) sampling of VH using bovine VH (BVH) as a model matrix. The meter was compared to an accredited Siemens Advia XPT Glucose Oxidase (GO) Assay and the Sigma-Aldrich BHB enzyme-linked immunosorbent assay (ELISA).

Methods
Using BVH and human serum albumin (HAS) as comparative matrices, method validation of the Abbott meter for both glucose and β-ketone (BHB), including linearity, bias, precision and interference was performed against current routine laboratory methods. Furthermore, application of the meter to a small cohort of procurators fiscal cases referred for post-mortem analysis was also assessed.

Results
The meter glucose assay was linear (curve fit: y = mx + c) over the analytical range 0.5-100 mmol/L in both HAS (inter-assay (n = 5) data: r² for slope of calibration curve (r²m) 0.9908; 0.46% relative standard deviation (RSD)) and BVH (inter-assay (n = 5) data: r²m 0.9927; 0.91% RSD), with no significant difference in correlation compared to gravimetric values (HAS: z = 1.67, p = 0.095; BVH: z = -0.37, p = 0.711). Imprecision at the accepted LLOQ for glucose (2.0 mmol/L) was 4.9% in HAS and 8.4% in BVH respectively. Intra-assay (n = 5) imprecision for glucose in HAS was 0.24% RSD (r²m 0.9841) and in BVH was 0.09% RSD (r²m 0.9972). The meter glucose assay demonstrated good correlation with the laboratory GO assay (Pearson’s r = 0.99) and Bland-Altman analysis indicated the analytical range of the meter had a non-significant (n = 20; Student’s T: p = 0.187) positive bias of 1.87 mmol/L measuring glucose (up to 20 mmol/L) in BVH. No significant difference (z = 0.01, p = 0.992) was observed between matched glucose standards measured in HAS and BVH by either the meter (n = 40; Student’s T: p = 0.692) or the laboratory method (n = 50; Student’s T: p = 0.387). No potential interference from other carbohydrates, including galactose, maltose, mannose and sucrose spiked into BVH was observed. The Abbott meter β-Ketone assay was linear over the analytical range 0.5-5 mmol/L in both HAS (inter-assay (n = 5) data: r²m 0.9790; 1.65% RSD) and BVH (r²m 0.9872; 1.90% RSD), with no significant difference in correlation compared to gravimetric values (HAS: z = 1.02, p = 0.308; BVH: z = 0.93, p = 0.352). Imprecision at the accepted LLOQ for BHB (1.0 mmol/L) was 9.8% in HAS and <5% in BVH respectively. Intra-assay (n = 5) imprecision for BHB in HAS was 0.68% RSD (r²m 0.9879) and in BVH was 1.09% RSD (r²m 0.9761). The meter BHB assay demonstrated reasonable correlation with an ELISA BHB assay (Pearson’s r = 0.93) and Bland-Altman analysis indicated the meter had a non-significant (n = 24; Student’s T: p = 0.582) negative bias of -0.24 mmol/L measuring BHB across the analytical range of the assay in BVH. No significant difference (z = 1.36, p = 0.174) was observed between matched BHB standards measured in HAS and BVH by either the meter (n = 20; Student’s T: p = 0.286) or ELISA (n = 20; Student’s T: p = 0.708). Potential interference from other volatiles, including acetone, ethanol, methanol, isopropanol and ethylene glycol spiked into BVH was not observed. Application of the meter to routine forensic examination was assessed using vitreous humour samples from 46 procurator fiscal cases referred for biochemical analyses during the course of this study. Using levels of glucose and BHB reportedly associated with causing death, two indices were defined: (1) a glycaemia index = ([Glucose]PATIENT – 13)/13 and (2) a ketosis index = ([BHB]PATIENT – 2.4)/2.4 , where 13 and 2.4 represent severe levels (mmol/L) of glycaemia and ketosis. By plotting the indices results for these patients using the data obtained from the meter, individuals whose cause of death had been independently attributed to diabetic ketoacidosis (DKA) were found to be easily categorised.

Discussion

Conclusions
The Abbott FreeStyle Precision Pro Glucose/β-Ketone Meter was shown to be an advantageous device, quickly and simply utilising small sample volume to assess severe glycaemia and ketosis in post-mortem vitreous humour. This study has demonstrated the potential benefit of POC instruments to forensic examination.
**Abstract ID 248**

**Suspected intoxication with nitrite: quantification of nitrite and nitrate in post-mortem human matrices.**

**Lauriane Drouin**

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**Aim**  
Sodium nitrite has various applications, such as a food additive in processed meat and medically as a third line treatment of cyanide poisoning in combination with sodium thiosulfate. Nitrite and its metabolite nitrate are endogenous compounds as well. Endogenous nitrite and nitrate plasma concentrations are < 0.01 - 0.8 mg/L and 0.6 - 6 mg/L respectively. Average nitrite and nitrate urine concentrations are 0.2 and 61 mg/L respectively.

In 2018, oral ingestion of nitrite was suspected to be the cause of death in two forensic cases in the Netherlands. The toxicity mechanism of nitrite is twofold. First, nitrite is reduced in vivo to nitric oxide, causing vasodilation and subsequent hemodynamic instability. Second, nitrite induces methemoglobinemia and subsequent hypoxia. Ingested doses of more than 4 g are potentially lethal.

An analytical method was developed to quantify nitrite and its metabolite nitrate in biological matrices (including postmortem whole blood and urine) and powder form.

**Methods**  
Sample preparation is based on the method from Yang et al (2013) with a few modifications (sample volume, temperature, evaporation protocol). Samples are precipitated (diluted for powder) with acetone and derivatised with pentafluorobenzylbromide (PFBBr) using tetraoctylammonium bromide as a phase transfer catalyst. The derivatised products of nitrite and nitrate are extracted with iso-octane. They are separated and analysed with GC-MS in single ion monitoring (SIM) mode.

Because nitrite and nitrate are endogenous compounds, the quantification is performed with calibrators prepared in deionized water, and the quality of the method is monitored using external controls prepared in deionized water. Analog (15)N-labeled internal standards are used for the quantification of nitrite and nitrate.

**Results**  
The calibration curve is linear between 5.0 mg/L and 50 mg/L. The lower limit of quantification (LLOQ) is 5.0 mg/L. The bias of the method is within 15%, and the repeatability within 16%. The recovery of the quality controls lies within 90%-110%.

The method has been applied to two forensic cases. In the first case, nitrite was detected in powder (646 g/kg) and in urine (lower than 50 mg/L) but not in blood, whereas nitrate was detected in blood (571 mg/L) and urine (85 mg/L), but was not detected in powder. In the second case, nitrite was detected in stomach content (4.1 g/L) and in heart blood (lower than 25 mg/L). Nitrate was detected in stomach content (141 mg/L) and in heart blood (273 mg/L).

**Discussion**  
The method is linear and shows an acceptable accuracy and recovery. It allows the separation of nitrite and nitrate, although its specificity in regards to other (fragments of) isobaric compounds was not completely verified. The nitrate blood concentration in all cases was higher than 50 mg/L. Therefore, it would be interesting to increase the concentration range of nitrate for its quantification in human matrices, in order to limit the extra dilution steps.

In our cases nitrite was almost completely oxidized to nitrate in blood samples, whereas it could still be found in high concentrations in the stomach content. Nitrate was also detected in the urine sample, even though its concentration is lower than that of nitrate.

**Conclusions**  
In case of a suspected death due to ingestion of nitrite powder, it is important to quantify both nitrite and nitrate in blood and urine. The analysis of stomach content is also a good indicator of a possible oral intake of nitrite. The method has been successfully applied to the two described forensic cases, and will be used after further optimisation and validation to be fit for purpose in future cases.
Abstract ID 249
A case of death after the consumption of N-Ethylpentylone and MPHP.

Cláudia Margalho
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Aim
Synthetic cathinones represent a large group of new drugs of abuse, which are increasing in popularity worldwide. This is justified because they are readily available in clandestine drug markets and because they are not detected by routine drug testing. N-ethylpentylone (also known as ephylone) and the 4′-methyl-α-pyrrolidinohexanophenone (MPHP) are two designer drugs with stimulant properties and with scarce available information related to their toxicology and pharmacology. We present a case of a 26-years old man, known as drug addicted, with hospital admission for detoxification treatment of opioid substances. He was being treated with the antidepressant (mirtazapine), the benzodiazepine (clonazepam), the antipsychotic (quetiapine) and the opioid antagonist (naltrexone). Internal examination of the body, by the forensic pathologist, revealed generalized visceral congestion and fluid blood. As cause of death there was a suspicion of intoxication by opiate substances.

Methods
Toxicological analyses were performed in peripheral whole blood, urine and vitreous humor. The analyses of drugs of abuse and benzodiazepines were first performed in blood by immunoassay procedure and the screening of ethanol was made by GC-FID-HS. After solid-phase extraction, (whole blood and vitreous humor) and liquid-liquid extraction (urine), a general screening for unknown substances was performed by GC-MS. The screening for prescription drugs was performed in blood by LC-MS-MS.

Results
The blood sample did not reveal the presence of classic drugs of abuse (opiates, cocaine and metabolites, cannabinoids, amphetamines and methamphetamines) by immunoassay screening and alcohol was negative. A range of prescription drugs were confirmed and quantified in blood by LC-MS-MS: mirtazapine (166.0 ng/mL), quetiapine (963.0 ng/mL), clonazepam (8.0 ng/mL) and paracetamol (500.0 ng/mL). The GC-MS screening revealed the presence of N-ethylpentylone and MPHP in blood, urine and vitreous humor. Subsequently, these two synthetic cathinones were confirmed and quantified in the blood and in the available alternative biological samples (urine and vitreous humor). The concentrations found in blood, urine and vitreous humor were respectively 42.0 ng/mL, 1220.5 ng/mL and 47.8 ng/mL for N-ethylpentylone; and 234.0 ng/mL, 3623.6 ng/mL and 214.6 ng/mL for MPHP.

Discussion
Conclusions
The potential effects of new psychoactive substances (NPS) remains a mystery to the health care professionals who still do not recognize the signs and symptoms of intoxication by this type of substances. Taken together, the results demonstrate that N-ethylpentylone and MPHP, can induce severe intoxication with fatal outcome. To the best of our knowledge, the case reported is the first fatality involving a combination of N-ethylpentylone and MPHP.
Abstract ID 252

Be aware of a barbiturate intoxication.

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Aim
In the past several years, the number of suicide attempts in the Netherlands has increased. The majority of these attempts involve the use of medication. Pentobarbital is one of the recommended drugs to end one’s life in a peaceful and reliable manner according to several websites. Pentobarbital can be easily obtained online through different websites, and with the help of several Dutch organizations such as: Foundation De Einder and Dutch society of voluntary euthanasia (NNVE). In order to bypass drug laws, pentobarbital is often labelled as a different substance, such as ‘skin cleanser’. Five cases of pentobarbital intoxications were presented at the Erasmus MC hospital. Four of the (postmortem) cases concerned successful suicide attempts where the persons had died in their homes or hotel. Intake of pentobarbital was suspected by the forensic doctors because of the presence of an unknown liquid and/or a urine test that indicated the presence of barbiturates. The fifth case concerned a person who was admitted to the emergency room in a critical sedated condition after a suicide attempt, with no indication which substance was taken. The patient survived after being treated in the intensive care unit (ICU). The aim is to increase the awareness of the possibility of a barbiturate intoxication in forensic and clinical toxicology.

Methods
Depending on the type of sample that was received, different analyses were performed. A drugs of abuse screening using immunoassay was performed on urine, when available. Pentobarbital analysis was performed on femoral blood and unknown liquids, using a validated HPLC-DAD method. Toxicological screening was performed on the urine and blood samples using a validated LC-MS/MS method.

Results
In the first postmortem case, urine of the deceased and two bottles of ‘skin cleanser’ with an unknown liquid were received. The urine was found positive for barbiturates, the unknown liquid contained pentobarbital with a concentration of 70 g/L. In the second postmortem case, two empty bottles labelled ‘Natural cosmetics’ were found near the deceased, blood of the deceased and an unknown liquid from a glass were received. Plasma of the deceased was found positive for pentobarbital with a concentration of 45 mg/L and the unknown liquid was found positive for pentobarbital with a concentration of 68 g/L. In the other two cases, blood of the deceased were obtained and showed plasma pentobarbital concentrations of 18 mg/L and 12 mg/L. Pentobarbital concentrations above 10mg/l are associated with serious toxicity. The toxicological screening of the urine and plasma of all these cases showed the presence of an antiemetic, either domperidon or metoclopramide. Urine of the fifth patient who survived the intoxication, was found positive for barbiturates and plasma was found positive for pentobarbital with a concentration of 9 mg/L.

Discussion
In 2018 and the beginning of 2019 five different cases were presented at the Erasmus MC hospital concerning pentobarbital intoxications, which are presented in this abstract. Four of the five cases were postmortem. An barbiturate intoxication does not show very often at the emergency department, probably because it is a very effective way of committing suicide. Since we are analyzing blood and urine in post mortem cases, in order to find more information about the cause of death, it has been clear that barbiturate intoxications do exist more than we expected. There are many options to get advise about using barbiturates to commit suicide. In the four post mortem cases we found an antiemetic as well, these are recommended to be taken 24 hours prior to the pentobarbital liquid intake to prevent emesis caused by pentobarbital intake. In two of the four post mortem cases pentobarbital was disguised as a different substance using bottles with labels such as ‘skin cleanser’, probably in order to make the shipment less suspicious.

Conclusions
Health care providers should be aware of an increased risk of pentobarbital intoxications, since it can be easily obtained online, and is stated to be the recommended drug when committing suicide on several websites. When a suicide is suspected, it is very important to take blood and urine samples (when available) and subsequently to look for any remaining liquid in bottles or glasses near the deceased in order to confirm the intake of pentobarbital.
**Abstract ID 256**

**Homicidal Poisoning Disguised as a Motor Vehicle Accident.**  
Amanda Jenkins

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**Aim**

The use of poisons to commit homicide is rare in the United States (US) and worldwide. In the last 20 years less than 0.20% of homicides committed in the US were attributed to poisons. In these cases medications/drugs were the most common agents. Other chemicals including arsenic, corrosive agents, carbon monoxide, strychnine, cyanide, and pesticides have been identified.

The objective of this presentation is to describe the investigation of a sudden death in Cuyahoga County, Ohio (OH), US, and to outline the multidisciplinary approach which permitted resolution of this case.

**Methods**

A 38 year Caucasian female was operating her motor vehicle when she became ill and collapsed at the wheel. The vehicle travelled left of center striking another vehicle. The victim was transported to the emergency room of a nearby hospital in full cardiopulmonary arrest. Advanced cardiac life support and drug therapy were instituted without success.

An autopsy was performed at the Office of the Cuyahoga County Coroner in Cleveland, OH, US, and routine toxicological testing included volatiles by headspace gas chromatography (GC) with flame ionization (FID); acetaminophen, salicylate and ethchorynol screening by colorimetry; benzodiazepines by liquid-liquid extraction followed by GC with electron capture detection; acidic-neutral drug screen by liquid –liquid extraction followed by GC with electron capture detection; acidic-neutral drug screen by liquid –liquid extraction followed by GC-FID and an alkaline drug screen by liquid –liquid extraction followed by GC-nitrogen phosphorous detection. Subsequent to the autopsy and investigation, additional testing was performed to include carbon monoxide, cholinesterase, cocaine and metabolites by GC-mass spectrometry (GC/MS), cyanide, digoxin, fentanyl, glycols, heavy metals, opiates by GC/MS and pesticides.

**Results**

Autopsy findings were unremarkable. There was no evidence of recent injury. Toxicological testing revealed a postmortem heart blood specimen contained cyanide at a concentration of 9.1 mg/L. Gastric contents were also positive for cyanide. Analysis was performed using Cyantesmo paper and Ion Selective Electrode. No other chemicals were detected in postmortem specimens.

Investigation revealed that the decedent had telephoned a friend while driving stating she did not feel well and that she had ingested a calcium capsule prior to leaving home. Law enforcement officials confiscated the capsules and they were tested at the Lake County Crime Laboratory, Painesville, OH, and also at Cleveland State University Chemistry Department. Testing included visual and microscopic examination, solubility, pH, GC/MS and Fourier Transform Infrared Spectroscopy, Spectrophotometry, X-Ray Fluorescence, Inductively Coupled Plasma Mass Spectrometry and 13C Nuclear Magnetic Resonance Spectrometry. Analysis of the calcium capsules determined that nine of the 56 capsules contained cyanide. The Coroner ruled the Cause of Death, Acute Cyanide Intoxication, and the Manner, Homicide.

**Discussion**

The husband of the deceased became the suspect in this case but he fled the US before he could be arrested. An international search ensued which included the US Federal Bureau of Investigation, US Marshals Service and Interpol. He was detained in Cyprus approximately 18 months later and extradited to the United States to stand trial. He was convicted of aggravated murder and sentenced to life in prison with parole eligibility after 20 years.

**Conclusions**

This case illustrates the importance of thorough medico-legal death investigation and the necessity of forensic toxicology laboratories able to perform or have access to a wide variety of testing. They should be aware of compounds that are not commonly observed in routine practice.

Collaboration between multiple forensic disciplines and agencies resulted in the determination of the cause and manner of death.
Abstract ID 267
The first cases of death involving the novel synthetic cannabinoid 5F-Cumyl-PEGACLONE.

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Aim
Aims: On the market for new psychoactive substances (NPS), synthetic cannabinoids (SCs) have continuously been introduced in the attempt to circumvent legal restrictions. Here we report three cases of death involving 5F-Cumyl-PEGACLONE, an emerging γ-carbolinone derived SC. 5F-Cumyl-PEGACLONE has a similar binding affinity as its non-fluorinated analog and currently appears to have a high relevance on the German drug market.

Methods
Methods: Death scene investigation (DSI) and post-mortem examination were performed, including histological analyses. Post-mortem samples of blood, urine, stomach content, hair, vitreous humor and tissues (brain, liver, kidneys) were initially screened by immunoassay, GC-MS and/or LC-MS/MS for common drugs of abuse and NPS. For quantification, toxicological analyses were performed using a validated LC-MS/MS method. When retrieved at DSI, herbal blends were analysed by GC-MS. Toxicological Significance Score (TSS) was assigned to the compound, in each case of death.

Results
Results and Discussion: All cases displayed non-specific signs of intoxication. In case 1, post-mortem examination revealed signs of hypothermia and acute kidney injury (AKI). A concentration of 0.22 and 2.4 ng/ml of 5F-Cumyl-PEGACLONE was measured in central and peripheral blood, while other drugs were each within their typical therapeutic ranges. A long-term history of SC use was confirmed by hair analysis. In case 2, a fresh injection mark, a severe pulmonary oedema and the high concentrations of opioids in blood and urine allowed to conclude that an acute, potentially fatal heroin intoxication occurred. In case 3, a prisoner was found dead, and in his cell herbal blends and paraphernalia for smoking were retrieved. GC-MS analysis of the herbal blend and the paraphernalia confirmed the presence of 5F-Cumyl-PEGACLONE.

Discussion
In case 1, despite a possible tolerance, the consumption of 5F-Cumyl-PEGACLONE as the cause of death seemed likely, thus a Toxicological Significance Score (TSS) of 3 was assigned. Moreover, a harmful effect of the fluorinated compound on kidneys is likely. In case 2, 5F-Cumyl-PEGACLONE, found in a concentration of 0.79 ng/mL, may be considered as an additional factor in the course of the intoxication (TSS=1). In case 3, the role of 5F-Cumyl-PEGACLONE in death was deemed as probable (TSS=2), despite the low concentration detected in femoral blood (0.030 ng/mL).

Conclusions
Conclusion: Despite deaths involving SCs have increasingly been reported, the assessment of the toxicological significance is still challenging. In the cases reported, probable fatal concentrations were lower than in the case where 5F-Cumyl-PEGACLONE only contributed to death. Thus, a comprehensive analysis of circumstantial, clinical and post-mortem findings, as well as in-depth toxicological analyses, are necessary for a valid assessment and interpretation regarding the cause of death.
Abstract ID 273
Potential effects of the co-administration of AMB-FUBINACA and pFPP in New Zealand

Diana Kappatos
Samantha Coward, Diana Kappatos
ESR

Aim
Since May 2017 more than 80 deaths across New Zealand (NZ) have been linked to the use of the synthetic cannabinoids AMB-FUBINACA and 5F-ADB. These new psychoactive substances have also been the most prevalent synthetic cannabinoids intercepted at the border by NZ Customs, and were detected in plant material submitted by NZ Police. A distinct geographical distribution of these drugs was observed across the country.

The geographical differences were also observed in intoxication cases, with AMB-FUBINACA associated with cases in the northern region of New Zealand’s North Island, and 5F-ADB more observed in the lower half of this island. The effects of the use of these synthetic cannabinoids was found to be rapid and profound, with severe adverse reactions such as seizures and unconsciousness, and death, occurring very quickly.

Interestingly, during analysis of the plant material submitted by NZ Police, ESR found that a proportion of the AMB-FUBINACA positive plant materials, also contained the stimulant para-fluorophenylpiperazine (pFPP). This was not present in plant material containing 5F-ADB. We do not fully understand why pFPP is present alongside AMB-FUBINACA, but it is possible that it was deliberately mixed into the plant material to help manage the severe effects associated with using AMB-FUBINACA on its own. Users of pFPP have reported a delay in the onset of the psychoactive effects after its use.

This study was undertaken to determine the potential effects of co-administration of these two psychoactive substances.

Methods
Biological specimens collected from users of AMB-FUBINACA were analysed by liquid chromatography with tandem mass spectrometric detection (LC-MSMS) and by liquid chromatography with time-of-flight (LC-TOFMS) for the presence of pFPP and a range of synthetic cannabinoids and some of their metabolites. The concentrations of pFPP and the synthetic cannabinoids were determined in the blood specimens.

Results
We have undertaken toxicological examination of blood and urine samples associated with users of AMB-FUBINACA and found pFPP in a number of cases. The levels of AMB-FUBINACA and AMB-FUBINACA metabolite in the blood of persons whose death appeared to be linked with AMB-FUBINACA were compared to those users who were either treated in emergency departments or had been found to be impaired drivers.

AMB-FUBINACA was found to be significantly metabolised in biological samples with a relatively small amount of the parent drug present in the blood and urine, whereas, pFPP itself was detected in the blood and urine. A significant number of cases whose death appears to be linked with AMB-FUBINACA had also used pFPP. The results of this study will be presented.

Discussion
Conclusions
Abstract ID 292
Development and validation of an analytical method for volatiles with endogenous production in putrefaction situations.
Carla Monteiro
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Aim
Considered the most identified psychoactive substance in postmortem toxicology, ethanol plays a major role in suicide, traumatic deaths, drowning, fatal accidents and violent crimes (e.g., facilitated violations). The possibility of endogenous ethanol production after death, which increases with the increase of temperature and the time elapsed between death and autopsy, is an issue for the correct interpretation of the analytical results obtained during a post-mortem analysis. In this way, the distinction between ante-mortem ethanol intake and its post-mortem production is often made through the corroboration of the results obtained in several matrices (e.g. urine and vitreous humor). According to the literature, the analysis of other volatiles formed during the putrefaction process allows to confirm the origin of the ethanol detected. The authors present the results obtained in an analytical method validation, in order to prove its adequacy in the detection and quantification of volatile substances (Ethanol, Acetone, 1-Pentanol, 3-Methyl-1-Butanol, 2-Methyl-1-Butanol, Acetaldehyde, 2-Propanol, Ethyl Acetate, Methyl Ethyl Ketone, 2-Butanol, Methanol, t-Butanol, Diethyl Ether, Isobutanol, 1-Butanol and 1-Propanol) in several biological samples (blood, urine and vitreous humor). After validation, the method was applied in different situations (e.g. putrefaction, submersion) in order to demonstrate its applicability.

Methods
For the accomplishment of the present study, a Varian 450-GC gas chromatograph with flame ionization detector, coupled to a headspace injector (HS-GC/FID) was used. Sample preparation was done by dilution of 100µL of the interest sample (e.g. biological samples, calibrators, quality controls) in 1mL of internal standard (Acetonitrile (0.1g/L)), having been used two capillary columns (VF624MS and VF5MS) with different polarities to ensure that all of the compounds under study were properly identified and undoubtedly distinguished from the remaining. All the volatiles were studied in a range of 50-2000mg/L and studied in terms of detection and quantitation limits, linearity, stability, carryover, precision, accuracy and specificity, according to the Scientific Working Group for Forensic Toxicology.

Results
The limits of detection (LOD) ranged between 1 and 8 and the limit of quantitation between 4 and 24. For the intermediate precision, a coefficient of variation of less than 10% was obtained for most substances. Although for Acetaldehyde, Diethyl-Ether and Methanol (100mg/L) the CV% was higher than this value, it remained below 20%. Relatively to intra-day precision, the obtained coefficient of variation was ≤6.4% and the bias was within the ±10% of the nominal concentration, fulfilling the required criteria. Finally, the method showed great selectivity at the concentration of 100mg/L and all volatiles showed linearity in the working range, with a correlation coefficient greater than 0.99. In the end of the study, the method was applied to 30 real samples with putrefaction and/or submersion information, positive for ethanol and outside the legal analysis period. During the qualitative analysis, all the substances under study were detected in the different matrices, having been acetaldehyde found in all of them. Furthermore, all the compounds were detected together in one case. This way, the results showed that the aim of the study was accomplished. After that, the quantitative method was applied and positive results were obtained for Acetaldehyde (between 60 and 180mg/L), 1-Butanol (100-500mg/L), 1-Propanol (50-100mg/L), 2-Propanol (80-120mg/L), Methanol (90-140mg/L) and Acetone (260-280mg/L).

Discussion
In general, all the results were quite satisfactory and fulfilled the imposed requirements successfully. Moreover, the fact that the running time is only 13 minutes is a great advantage since it leads to obtaining results for a several substances in a short time interval.

Conclusions
The method developed proved to be a great advantage for application in the context of forensic toxicology since, in addition to allowing the study of 16 volatiles, it also allows the use of reduced sample volumes, which is an aspect of great importance, since the amount of sample available for analysis is often reduced. Finally, the presented method is a complementary one so it cannot be used as a single analysis.
Abstract ID 296

4-Methylethcathinone Related Death of a Slammer Documented with Postmortem Concentrations.

Jenny Becam

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Aim
To present fatality related to 4-MEC in a subject practicing slam and to discuss the concentrations measured in the various postmortem toxicological samples.

Case Report. A 47-year-old male was found dead at his home at the end of the night. His cohabiting partner explained to the investigators that they had administered themselves between 6 pm to 5 am a dozen of intravenous shots of “GHB and methamphetamine, called 3-MMC or 3-MEC”, followed by sexual intercourse. He then went to bed, and when he woke up, found his partner lying on the floor in the living room. He practiced a cardiac massage on the victim until rescue services arrived. Death was pronounced in the late morning after 30 minutes of resuscitation. When lifting the body, two syringes, a steribox packaging, a stericup packaging, and an almost empty bag marked “3-MMC”, unknown to the partner, were found, suggesting that the victim could have had one last injection alone while his companion was asleep. Among the victim’s past history, the notion of precordial pain is being explored. The autopsy revealed recent injection site stigmas in both arms, diffuse polyvisceral congestion, acute pulmonary oedema, and advanced coronary artery disease with cardiac hypertrophy. The usual toxicology test samples (cardiac blood (CB), peripheral blood (PB), gastric content (GC), urine (Ur), vitreous humor (VH), liver (Li) and lungs (Lu)) were taken. The victim being shaved, no removal of head or facial hair could be done.

Methods
Alcohols were assayed by HS-GC / FID. Measurement of the HbCO was carried out by spectrophotometric method. A broad screening for drugs and narcotics, including NPS, was performed by LC-HRMS. The specific search for narcotic substances and GHB was carried out on all the samples by LC-MS / MS.

Results
Alcohol determination showed the presence of ethanol at a concentration of 9 g/L in the gastric contents. HbCO was 2.8%. The search for NPS by LC-HRMS showed the presence of 4-MEC in the various samples at the following concentrations (μg/L): CB: 2450; PB: 4890; Ur: 6560; VH: 838; GC: 1690; Lu: 400; Li: not detected. Other investigations, including the search for GHB, were negative.

Discussion
Slam is a practice used in chemsex and involves injecting intravenous molecules into sexual intercourse in order to multiply the effects. This practice is common in the MSM (male who have sex with male) population. While cocaine and methamphetamine have often been used in this context, various cathinones, including 3-MMC and 4-MEC, have replaced these molecules because of their better accessibility and lower cost. In this case, it must be noted that the drugs found in the postmortem samples did not correspond to those indicated on the labels of the bottles and bag (which could explain the absence of GHB in the samples) and that there was confusion from the users between amphetamines and cathinones. The cause of death is a 4-MEC overdose in a victim with severe coronary artery disease, which may have contributed to the death. The concentrations measured in the present case are compatible with those few described in the literature (Smith PR et al. Reporting two fatalities associated with the use of 4-methylethcathinone (4-MEC) and a review of the literature. Toxicol 2016; 40: 553-560; Allard S et al. « Chemsex »: 2 cas de décès. Toxicol Anal Clin 2017;29(2):S21). It is difficult to document a possible postmortem redistribution between different blood compartments in this case. Indeed, the victim underwent a prolonged cardiac massage, which frequently leads to a redistribution of the molecules from the cardiac cavities to the peripheral blood. Although the substance has been administered intravenously, the presence of 4-MEC in the gastric contents probably comes from a postmortem redistribution phenomenon. The concentration of 4-MEC in vitreous humor is much lower than that measured in blood samples. This result is not in accordance with those described by Baccati et al. Finally, the high urinary concentration is in accordance with these previous published data (Baccati C et al. Éléments de distribution post-mortem de la 4-méthylethcathinone et du métabolite déséthylé : à propos de deux décès. Toxicol Anal Clin 2018;30(2):S22-S23).

Conclusions
Non-detection of 4-MEC in the liver remains an enigma and it will be necessary to collect other cases to better document the postmortem redistribution of this substance which is gaining popularity in the MSM (male who have sex with male) population practicing chemsex.
Abstract ID 304
Evaluation of CO-Hb quantification in post-mortem whole blood by oximetry and headspace gas chromatography with flame ionization detection.

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Aim
To compare the oximetry and HS-GC-FID methods for CO-Hb quantification in post-mortem whole blood samples.

Methods
The Department of Forensic Sciences at Oslo University Hospital (Oslo, Norway) annually receives approximately 2000 whole blood samples from forensic autopsies. In about 100 of these samples carboxyhemoglobin (CO-Hb) analysis was requested based upon suspicion of CO intoxication. In about 50% of these cases, CO was found at a concentration above 5% CO-Hb. According to forensic toxicology guidelines of Society of Forensic Toxicologists (SOFT), screening and confirmation methods should, whenever possible, be based on different chemical principles in two independent extracts. Based on this principle, two different methods have been used at our department for determination of CO-Hb. Screening was performed with oximetry measuring at 128 different wavelengths, whereas confirmation and quantification was performed with determination of CO converted to CH4 using a catalyst and headspace gas chromatography with flame ionization detection (HS-GC-FID), and determination of Fe with atom absorption.

Oximetry (ABL80, Radiometer): About 100 µL whole blood was loaded into the oximetry module. %CO-Hb and %Met-Hb was determined.

HS-GC-FID analysis (Triplus 300 HS and Trace 1300 series GC-FID, Thermo Scientific): One mL whole blood was added 3 mL saponin solution, mixed and centrifuged for 20 min, 3000 rpm at 6°C. To 20 mL HS-vials, 400 µL of the supernatant was added and the vials were immediately sealed. One mL potassium hexacyanoferrat (III)-solution was added through the HS-septum and mixed. The samples were termostated at 70°C for 5 min. CO was separated using He as carrier gas and a CP-Molsieve column (5Å, 30 m, 0.32 mm, 10.00 µm, Agilent). CO was determined after catalytic reduction of CO to CH4 before FID detection. Fe analysis (Varian SpectrAA 50): 400 µL of the saponin supernatant was diluted to 10 mL with Type-1 water (Milli-Q). Fe was determined at 248.3 nm. The %CO-Hb content was calculated from the Fe-concentration of the sample and the amount of CO, using the fact that 99% of Fe in whole blood is bound to Hb, and that 100 µg of Fe-Hb under normal physiological conditions can bind 40 µL CO, according to Iffland and Sticht (Archiv. Toxicol. 29, 325-330, 1972).

Results
During a period of about 3 years, 124 post-mortem whole blood samples were analysed on both methods, one replicate on each method. The authentic samples were of varying quality and some were putrefied, carbonized or coagulated. The method comparison showed good correspondence between the %CO-Hb found by either oximetry analysis, or HS-GC-FID combined with atom absorption analysis, for the whole concentration range (5% to about 85% CO-Hb), with slope 1.02 and R2 0.97 using linear regression. Five samples, all with %CO-Hb > 40%, showed deviations from the confirmation method of more than 10% CO-Hb in absolute terms. Three samples were lower on the oximetry method, range -20 to -11 %CO-Hb, all of these samples were diluted with water. Two samples showed a +15% deviation, and during analysis there were no comments indicating problems for these samples. The methods showed satisfactory results in proficiency testing rounds, with z-scores < ±2 (n=11).

Discussion

Conclusions
The oximetry and the HS-GC-FID methods are comparable and satisfactory for %CO-Hb determination in forensic post-mortem whole blood samples. The HS-GC-FID atom absorption method was time and sample consuming, and also demanded dedicated instrumentation. Based on this, our department now uses the oximetry method for both screening and confirmation of CO-Hb.
Abstract ID 308
The flood of “yellow heroin” (high-potency heroin) and the increase of heroin-related overdose deaths in north-east Italy during 2017-2018.
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Aim
During 2017-2018 there was an impressive increase of high-potency heroin (HPH) availability at street-level in north-east Italy. Such HPH was termed by local media “yellow heroin”. Fatal and non-fatal heroin-related overdoses consistently increased in the same area during the above period. Authors’ laboratory was involved in toxicological analyses of both seized drugs and autopsy specimens in case of overdose deaths. The presentation reports on the relative findings, comparing the data of the period Jan 2017- Feb 2018 with those of a previous three-year period (2014-2016).

Methods
A total of 889 heroin samples, seized by police during both the 2014-2016 and Jan 2017- Feb 2018 periods, were analyzed at the authors’ laboratory, on request of prosecutor’s offices of six north-east Italy provinces. Toxicological analyses were also carried out on autopsy specimens (blood, urine, etc.), paraphernalia (residues from syringes, spoons, vials, envelopes, etc.), and drug doses found at the death scenes, in relation to 17 overdose deaths occurred through the above periods, classified as heroin fatal overdoses.

Results
The heroin content was less than 10% in 693 out of the total 889 seizure samples (78%), while it was in the range 10-74%, with a mean about 30%, in 196 preparations (22%). These last samples were categorized as HPH seizures. Noteworthy, HPH seizures represented only 13.3% of the total heroin seizures in the three-year period 2014-2016, whereas they were 45.5% in the fourteen month period Jan 2017- Feb 2018. Thus, nearly half of the street heroin doses were HPH in the last period, with an increment of HPH diffusion of 238% with respect to the previous one. Moreover, a strict relationship between HPH seizures and the presence of the adulterant dextromethorphan (DXM) was observed. In particular, all seizures containing the opioid DXM were HPH seizures, hence DXM was considered a valuable chemical marker of HPH, at least in north-east Italy. No fentanyl, their analogues, or other opioids were found in both low-potency and HPH seizures. Heroin fatal overdoses were 7 in the three-year period 2014-2016 (0.2 per month), while they were 10 in the Jan 2017- Feb 2018 period (0.7 per month), with an increment of 250%. 8 out of these 10 deaths occurred from Jul to Sept 2017, with a rate of 2.7 per month. In 9 out of 10 deaths of the 2017-18 period DXM and its metabolite dextrorphan (DXR) were detected in autopsy specimens, as well as in the majority of paraphernalia residues. All drug doses found at the death scenes contained heroin in the range 30-40%, along with DXM. In some cases alcohol and other psychoactive/pharmaceutical drugs, although no opioids other than heroin and DXM, were detected in autopsy specimens. Conversely, HR-MS retrospectively processed full-scan data showed the absence of DXM/DXR in autopsy samples from the 2014-2016 period deaths.

Discussion
The availability at street-level of HPH was much more pronounced in north-east Italy during the period Jan 2017- Feb 2018 compared to the previous three-year period 2014-2016. Typical heroin contents of nearly half of the heroin doses of the most recent period were in the range 30-50%, opposite to before when typical doses were in the range 0.3-3%. Even though it is known that the overdose risk may be related to various parameters, including individual pharmacokinetic factors, health status, underlying comorbidities, the environmental context and poly-drug use, the HPH can be considered the prominent risk factor for acute toxicity, as the respiratory depression is directly proportional to the amount of the opioid consumed. Hence, the intake of HPH may easily drive overdose in both non-tolerant and tolerant users. The role of DXM, an antitussive drug at low doses, with dissociative/hallucinogen properties at higher doses, and capable to produce tachycardia, hypertension, and respiratory depression at massive doses, especially when administered intravenously, is worthy of being better investigated.

Conclusions
The flood of HPH at street-level during the period Jan 2017- Feb 2018, as well as the consistent increase of fatal and non-fatal heroin-related overdoses in the same area and time period, raised great concern in north-east Italy. Thus, significant law enforcement initiatives were implemented by judicial authorities to combat the HPH illicit trafficking. Furthermore, the HPH phenomenon led to a strengthened collaboration among hospital emergency departments, addiction treatment departments, harm reduction services, and toxicology laboratories. Luckily, a decreasing trend, but not the disappearance, of both HPH availability and heroin-related overdoses have been recently observed.
Abstract ID 318
Identification of acetyl-, acryl-, cyclopropyl-, isobutyryl- & 4F-isobutyrylfentanyl metabolites using in-house synthesized standards, hepatocytes & LC-QTOF-MS.

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Aim
Fentanyl analogs constitute an important group of new psychoactive substances (NPS) responsible for numerous deaths around the world during the last years. As such, it is important to study both their toxicodynamics and toxicokinetics, including their metabolism. NPS metabolism studies are normally conducted using in vitro model systems and/or authentic urine samples in combination with high-resolution mass spectrometry. While being very effective in providing high quality knowledge, the combination is limited in the ability to distinguish positional isomers, such as hydroxylations. Identifying the correct structures of the metabolites is important as metabolites may be active and contribute to the toxicity of fentanyl analogs. Getting the correct reference material is also important to uniquely identify an intake, investigate the detection windows and the toxicokinetics of a fentanyl analog.

Earlier metabolism studies have shown that biotransformations of fentanyl analogs include mono- and dihydroxylations as well as methylation on the phenethyl moiety. The aim of this study was to identify the exact structure of these metabolites by synthesizing potential structure analogs and compare with metabolites identified after hepatocyte incubations.

Methods
Based on earlier studies, seven motifs were selected for synthesis of fentanyl and the fentanyl analogs acetyl-, acryl-, cyclopropyl-, isobutyryl- and 4F-isobutyrylfentanyl. This included four monohydroxylated motifs on the phenethyl moiety (4-OH, 3-OH, 2-OH and β-OH), the catechol 3,4-diOH and the two different O-methylation products of the catechol of all the six different fentanyls.

All six parent drugs were incubated at 5 µM with cryopreserved hepatocytes, one million cells/ml, for five hours at 37 °C. The reactions were stopped by addition of acetonitrile and the supernatants analyzed by LC-QTOF-MS together with the in-house reference materials diluted in mobile phase.

The hepatocyte incubations and the 42 synthesized metabolites were analyzed using an Agilent 6550 QTOF coupled to a 1290 LC-system. The separation was achieved on an Acquity HSS T3 column (150 × 2.1 mm, 1.8 µm) with a matching guard column at 60 °C using a flow rate of 500 µl/min using different 13 min gradients of 0.05% formic acid in 10 mM ammonium formate and 0.05% formic acid in acetonitrile.

For acetyl- acryl- and 4F-isobutyrylfentanyl urinary metabolites could be identified by comparing with previous experiments using both hepatocytes and urine samples. Urinary metabolites were identified based on relative retention time, abundance after hepatocyte incubation, diagnostic ions and accurate mass. For cyclopropylfentanyl, 13 urine samples from cases were cyclopropylfentanyl was identified in femoral blood were analyzed together with the reference materials.

Results
For all analogs, β-OH, 4-OH and 4-OH,3-OMe were identified after hepatocyte incubation and for all analogs except acetylfentanyl β-OH was the major hydroxylated metabolite. Interestingly, in the urine samples, the major hydroxylated metabolite was 4-OH rather than β-OH for all analogs were urine sample data was available.

The catechol 3,4-diOH was only identified after hepatocyte incubation with acetylfentanyl at low abundance. However, while generally not observed after hepatocyte incubations it was shown to be a major metabolite in urine samples for all analogs.

3-OH,4-OMe was not detected in any hepatocyte samples indicating a clear preference for the 4-OH,3-OMe which was also found to be more abundant in urine compared to hepatocytes.

Discussion
Based on the results of this study we can firmly conclude that the β-OH, 4-OH, 3,4-diOH and 4-OH,3-OMe are major metabolites of the six investigated fentanyl analogous and could all be suitable as urinary markers of drug intake together with the corresponding normetabolites. As these metabolites are oxidations rather than dealkylations they are generally more selective markers as fewer analogs could produce an identical metabolite.

By showing that several structurally diverse fentanyl analogs have similar major metabolites, it is reasonable to suggest these motifs may also be relevant for many other fentanyl analogs, including novel ones not yet investigated. That said, earlier studies have shown that some analogs, such as furanylfentanyl have a very different metabolism.

The identity of major metabolites as well as the synthesis routes provided could serve as the basis for the manufacture of reference materials and the activity of the metabolites at the opioid receptor will be evaluated to investigate the role of metabolites in fentanyl analog toxicity.

Conclusions
By analyzing reference materials synthesized in-house of major metabolites of fentanyl and the fentanyl analogs acetyl-, acryl-, cyclopropyl-, isobutyryl- and 4F-isobutyrylfentanyl together with samples from hepatocyte incubations and data from earlier analysis of urine samples it was shown that β-OH, 4-OH and 4-OH,3-OMe are major metabolites of all fentanyl analogs studied. In urine samples, the catechol 3,4-diOH was also shown to be a major metabolite.
Abstract ID 327

Starvation suspicion in an enfant explored by postmortem biochemistry and testing for trace elements in hair.

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Aim
Present two complementary approaches to explore the nutritional status of an enfant who died with major stunting in a context of abuse.

A 9-months-old girl was found dead in the bed of her parents. Suspecting a sudden infant death syndrome, the rescue services has transferred the corpse to a specialized hospital unit. The first investigations (D1) consisted in clinical examination and blood sampling for bacteriology, virology, toxicology and genetics according to the recommendations (Bajanowski T et al. Sudden infant death syndrome (SIDS) – standardised investigations and classification: recommendations. Forensic Sci Int 2007;165:129-143). As the first examination showed multiple ecchymosis on the corpse, an inquiry has been opened and the corpse was transferred to the forensic institute. Autopsy with usual postmortem samples for toxicology (femoral blood, bile and hair) was done at D2 and revealed a complex parieto-occipital fracture with subdural hematoma, cause of death, as well as a severe growth retardation without any dysmorphism or malformation. In this context, the magistrate ordered, in addition to pathological and toxicological investigations, to carry out any analysis to evaluate the existence of dietary deficiencies or malnutrition.

Methods
Toxicological investigations were performed autopsy samples. In femoral blood were measured alcohols, including acetone, by HS-GC/FID. Femoral blood and hair were screened for pharmaceuticals, drugs of abuse and NPS by routine procedures including immunoassays, LC-DAD and LC-MS/MS. The two complementary approaches used in this case to explore the nutritional status are postmortem biochemistry and hair testing for trace elements. Biochemical investigations were performed in the serum from the blood sample taken on D1 and in the whole blood taken on D2. Albumin, pre-albumin, urea, creatinine and uric acid were measured in the serum and beta-hydroxybutyrate in whole blood according to previously described procedures (Palmiere C et al. Postmortem biochemistry in suspected starvation-induced ketoacidosis. J Forensic Leg Med. 2016;42:51-55). The testing for heavy metals and trace elements showed concentrations comparable to those measured in healthy subjects. These results are not in favor of a nutritional deficiency in the months preceding the death. The pathological examination confirmed the subdural hematoma and showed a slight renal tubules ectasia.

Results
All the toxicological investigations were negative. Biochemical analyses showed an increase of uric acid at 1014 µmol/L (N: 142-339 µmol/L), while albumin, pre-albumin, urea, creatinine and beta-hydroxybutyrate concentrations were within the normal range. These results allow to rule out a ketoacidosis as well as a dehydration at the time of death. The testing for heavy metals and trace elements showed concentrations comparable to those measured in healthy subjects. These results are not in favor of a nutritional deficiency in the months preceding the death. The pathological examination confirmed the subdural hematoma and showed a slight renal tubules ectasia.

Discussion
In the context of a growth retardation in a child victim of maltreatment, the exploration of the nutritional status is of medicolegal interest. The two complementary approaches allowed to rule out a starvation. The victim medical record, consulted as part of investigation, revealed an intrauterine growth restriction with a low birth weight. The increase in acid uric associated with the growth retardation could be related to a chronic kidney disease (Rhone ET & Carmody JB. Birthweight and serum acid uric in American adolescents. Pediatr Int 2017;59:949-950). The renal tubules ectasia discovered during the pathological examination is in favor of such a diagnosis.

Conclusions
In conclusion, associating postmortem biochemistry with the measurement of trace elements in the hair makes it possible to eliminate malnutrition at the time of death and to assess the nutritional state of the victim on the period preceding the death.
Abstract ID 328
Starvation suspicion in an enfant explored by postmortem biochemistry and testing for trace elements in hair.

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Aim
Present two complementary approaches – postmortem biochemistry and hair testing for trace elements – to explore the nutritional status of an enfant who died with major stunting in a context of abuse.

A 9-months-old girl was found dead in the bed of her parents. Suspecting a sudden infant death syndrome, the rescue services has transferred the corpse to a specialized hospital unit. The first investigations (D1) consisted in clinical examination and blood sampling for bacteriology, virology, toxicology and genetics according to the recommendations (Bajanowski T et al. Sudden infant death syndrome (SIDS) – standardised investigations and classification: recommandations. Forensic Sci Int 2007;165:129-143). As the first examination showed multiple ecchymosis on the corpse, an inquiry has been opened and the corpse was transferred to the forensic institute. Autopsy with usual post-mortem samples for toxicology (femoral blood, bile and hair) was done at D2. The autopsy revealed a complex parieto-occipital fracture with subdural hematoma, cause of death, and a severe growth retardation without any dysmorphism or malformation. In this context, the magistrate ordered, in addition to toxicological investigations, to carry out any analysis to evaluate the existence of dietary deficiencies or malnutrition.

Methods
Toxicological investigations were performed autopsy samples. In femoral blood were measured alcohols, including acetone, by HS-GC/FID. Femoral blood and hair were screened for pharmaceuticals, drugs of abuse and NPS by routine procedures including immunoassays, LC-DAD and LC-MS/MS. Biochemical investigations were performed in the serum from the blood sample taken on D1 and in the whole blood taken on D2. Albumin, pre-albumin, urea, creatinine and uric acid were measured in the serum and beta-hydroxybutyrate in whole blood according to previously described procedures (Palmiere C et al. Postmortem biochemistry in suspected starvation-induced ketoacidosis. J Forensic Leg Med. 2016 Aug;42:51-55). The testing for 15 heavy metals and trace elements (Mn, Cu, Zn, Se, Al, Sr, Ag, Sn, Sb, Ba, Hg, Ti, Pb, As, Bi) was carried out by ICP-MS in the hair strand taken during the autopsy (Kintz P. Hair and diet assessment. Testing for trace elements by ICP-MS. Ann Toxicol Anal. 2007;XIX(1):65-69).

Results
All the toxicological investigations were negative. Biochemical analyses showed an increase of uric acid at 1014 µmol/L (N: 142-339), while albumin, pre-albumin, urea, creatinine and beta-hydroxybutyrate concentrations were within the normal range. These results allow to rule out a ketoacidosis as well as a dehydration at the time of death. The testing for heavy metals and trace elements showed concentrations comparable to those measured in healthy subjects. These results are not in favor of a nutritional deficiency in the months preceding the death.

Discussion
In the context of a growth retardation in a child victim of maltreatment, the exploration of the nutritional status is of medicolegal interest. In this case, the two complementary approaches allowed to rule out a starvation. The victim medical record, consulted as part of investigation, revealed an intrauterine growth restriction with a low birth weight. At this time, the increase in acid uric remains unexplained.

Conclusions
In conclusion, associating postmortem biochemistry with the measurement of trace elements in the hair makes it possible to eliminate malnutrition at the time of death and to assess the nutritional state of the victim on the period preceding the death.
Abstract ID 340
Cases of poisoning or death related to suspicion of psychoactive substances or drugs abuse being analyzed at the forensic genetics institute in 2018.
Jakub Czarny
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Aim
The availability of numerous psychoactive drugs and medicines on the black market results in an increased number of poisoning and deaths associated with their intake. Growing interest in these substances makes it very important to develop methods for the determination of these compounds and their metabolites for routine diagnosis of poisoning or for analysis of the cause of death. Due to the number of psychoactive compounds available on the black market for routine poisoning diagnostics, a multiplex method of analysis of psychoactive compounds based on the LC-MS/MS method was implemented, which allowed for simultaneous analysis of 521 compounds.

Methods
Using certified standards of psychoactive substances, their metabolites and drugs, a method for their determination based on the MRM pair mode of monitoring was developed for particular compounds. The method uses: liquid chromatograph 2x Exion LC AC Pump, Exion LC Degaser, Exion AC Autosampler, Exion LC Column Oven coupled with a Sciex 5500 QTRAP mass spectrometer operated in ESI mode. Optimal separation conditions of the analytes were selected using a Kinetex C18 100 x 3 mm, 2.6 μm chromatographic column with a buffered methanol/water phase system. The parameters of the mass spectrometer was optimized for each of the analytes. Samples were prepared using liquid-liquid extraction. The method was validated for 549 analytes following the SWGTOX guidelines, and a positive validation result was obtained for 521 analytes.

Results
The study was based on 71 blood samples (in some cases urine) routinely analysed related to the suspicion of deaths or poisoning caused by the use of psychoactive substances at the Institute of Forensic Genetics in Bydgoszcz in 2018. In the group of 71 cases, 51 involved men, and 20 women. Only 28 of the tested blood/urine samples did not contain any of the analytes analyzed for the rest of our method. The study presents detailed information on individual cases, including information about the examined person (gender, age), the substance found and its concentration. In 43 of cases, the presence of compounds analyzed using our multimethod has been found. Sometimes it is not detected in routine screening tests. Detected compounds in the tested samples are: GHB, dihydrocodeine, doxepin, 2-fluoroamphetamine, 3-fluoroamphetamine, 2-fluoromethamphetamine, 3-fluoromethamphetamine, morphone, quetiapine, codeine, paracetamol, desmethylidazepam, diazepam, tramadol, estazolam, sertrialline, atenolol, 7-aminoclonazepam, α-clonazepam, methandienone, midazolam, pregabaline, alpha-hydroxymidazolam, 3-CMC, 4-CEC, 4-CMC, oxazepam, temazepam, alpha-ethylaminopentiophenone, PV8, hydrocodone, N-propylamphetamine, THC, EDDP, THC-COOH, mianserin, methadone, benzylamine, methamphetamine, amphetamine, 3,4-MDMA, 5-F-ADB, 5F-NPB-22, propranolol. In 36 cases more than one substance was detected.

Discussion
In most of the analyzed samples, at least one substance was detected and the concentration of identified drugs usually exceeded the therapies dose. There are many NPS among the substances listed above. However, despite the wide range of substances analyzed in 28 samples, we did not find any of them. Due to the lack of a clear cause of death, this situation may indicate that it may have been caused by another new psychoactive substances (NPS) that we do not have in our method. This shows that the method should be further developed to provide as much as possible of the explained causes of death.

Conclusions
The developed method for the determination of psychoactive substances, drugs and their metabolites, which meets the conditions of the confirmatory method according to the guidelines of SWGTOX, has been successfully applied to routine toxicological analyses. The presented results indicate that the developed method is a useful diagnostic tool considering the growing popularity of recreational use and abuse of a wide range of new psychoactive substances and drugs, but it is still insufficient and needs to be constantly expanded by meeting the expectations of forensic toxicology.
Abstract ID  360
A fatal case probably after an intramuscular injection of paliperidone.

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Aim
Trevicta® (paliperidone palmitate) is an antipsychotic drug which has obtained the European Marketing Authorization Application in 2016. This treatment consists in an intra-muscular injection (in deltoid or gluteal muscle) every three months. After a single injection, the plasma concentration of paliperidone increases till a maximum concentration (Cmax) reached at a maximum time of 30-33 days. The total exposure to paliperidone is proportional to the administered dose, ranging between 175 and 525 mg. The pharmacokinetics exhibits a large intra- and inter-individual variability. The most frequent side effects reported are headache, weight gain, nasopharyngitis, and akathisia [1]. Because of the recent availability of Trevicta®, only few fatal cases were documented. Hence, we reported a lethal intoxication after a single injection of Trevicta®.

Methods
Right femoral blood was sampled, and toxicological screenings were performed using HPLC-UVDAD. The sample preparation was performed using two methods of liquid/liquid extraction [2]. The injection volume was 10 µL and the analysis time was 20 minutes using a HP1100 HPLC system (Agilent Technologies). The column was an Uptisphere C18 ODB column (5µm, 100 x 2.1 mm, Agilent Technologies). Data acquisition was performed at three wavelengths (210, 230 and 254 nm) and products were identified by comparing their spectra and retention time with those recorded in the home-made spectrum library.

Results
In our case report, only paliperidone was detected and its blood concentration was of 240 ng/ml.

Discussion
After information from the psychiatrist, 525 mg of Trevicta® were injected in the deltoid muscle 14 days before. This concentration was higher than the Cmax published by Savitz et al. [3], ranging between 40 and 55 ng/ml after a single injection of Trevicta® 525 mg. After a deltoid injection of Trevicta®525 mg, a median Cmax of paliperidone of 57.9 ng/ml [27.6-416 ng/ml] reached at a median T_max of 24.5 days [1-55 days] and a median half-life of 56.9 days [21.3-115.2 days] were reported in 24 patients by Ravenstijn et al.[4]. This highlights the huge inter-variability of concentration of paliperidone. Furthermore, an additional variability is due to the site of injection: Cmax after injection in deltoid muscle is 11-12% higher than after gluteal muscle injection. The median apparent half-life ranged from 84 to 95 days after deltoid injection and from 118 to 139 days after gluteal injection.

After risperidone administration, the therapeutic range, which is the sum of risperidone and its metabolite (paliperidone or hydroxyrisperidone) concentrations, is less than 100 ng/ml. Concentrations higher than 120 ng/ml can be associated with toxic effects. Blood concentration higher than 1000 ng/ml is considered to be potentially lethal without medical care. In case of overdose, the signs are: drowsiness, sedation, tachycardia, hypotension, QT interval prolongation and extrapyramidal side effects. More severe effects were also described such as torsades de pointes and ventricular fibrillation.

The American database Poisindex has recorded 804 cases of intoxication after oral administration of paliperidone. Among them, a 23-year-old woman with schizophrenia antecedents who has ingested 55 tablets of 9mg paliperidone ER, 400 mg of quetiapine and 15 mg of zopiclone presented a sinusal tachycardia three hours later. The plasma concentrations of paliperidone were of 29 ng/ml 4 hours after ingestion and of 883 ng/mL 40h after ingestion. She was discharged after suitable medical care [5]. In Japan, 32 fatal cases were reported between November 2013 and May 2014 after paliperidone palmitate once-monthly injection [6].

Conclusions
Trevicta® is a recently marketed drug. This formulation of paliperidone is characterized by a large intra- and inter-individual variability and several cases of intoxication have been reported to date. Here, in our case, despite the lack of an autopsy, Trevicta® poisoning appears to be the most likely cause of death. This case highlights i) the necessity to always evaluate the benefit-risk balance when prescribing this sustained-release form of paliperidone, overall in a context of cardiac condition ii) difficulties in interpreting paliperidone concentration in cases of intoxication.

Abstract ID 362
The prevalence of alcohol and other drugs in fatal road crashes in Victoria, Australia.

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Aim
Driving under the influence drugs, including alcohol, is a globally recognised risk factor for road traffic crashes, leading to injury and death. While the prevalence of alcohol and other drugs in fatal road crashes has been examined in other countries, data studying the prevalence of alcohol and other drugs in road traffic crashes more recently in Victoria, Australia, is limited. This study therefore aimed to examine how the presence of alcohol and other drugs in fatal road trauma has changed over time and if this coincides with increasing drug use in the general population.

Methods
A population-based review of out-of-hospital and in-hospital road trauma deaths was performed over the period of 01 July 2006 to 30 June 2016 in Victoria, Australia, using data from the National Coronial Information System (NCIS) and the Victorian State Trauma Registry (VSTR). Passengers and occupants out of the vehicle were excluded. Toxicology data were linked from the Victorian Institute of Forensic Medicine. Nine drug classifications were used: alcohol, cocaine, amphetamine, ketamine, opioids, Delta-9-tetrahydrocannabinol (THC), benzodiazepines, antidepressants and antipsychotics. Individual Poisson regression models were used to determine whether the incidence rate increased or decreased over the study period for each road user group and for each drug type. The incidence rate ratio (IRR) and 95% confidence intervals (CI) were calculated.

Results
There were 2410 road traffic fatalities over the study period. These constituted 1399 (58%) motor vehicle drivers, 511 (21%) pedestrians, 417 (17%) motorcyclists and 83 (3%) pedal cyclists. Road traffic fatalities declined in motor vehicle drivers (IRR=0.95; 95% CI: 0.94, 0.97), motorcyclists (IRR=0.95; 95% CI: 0.92, 0.99) and pedestrians (IRR=0.94; 95% CI: 0.91, 0.97) but there was no change in pedal cyclists (IRR=0.98; 95% CI: 0.91, 1.06). A blood alcohol concentration ≥0.05 g/100mL was present in 18% of all road traffic fatalities. The prevalence of alcohol declined 7% per year in motor vehicle drivers (IRR=0.93, 95% CI: 0.90, 0.97) and 7% per year in pedestrians (IRR=0.93; 95% CI: 0.87, 0.99), but did not change in motorcyclists or pedal cyclists.

For all road traffic fatalities, opioids were detected in 16% of cases, in addition to THC (13%), antidepressants (9%), benzodiazepines (8%), amphetamines (7%), ketamine (3%), antipsychotics (1%) and cocaine (0.2%). In motorcyclists, the prevalence of opioids increased 12% per year (IRR=1.12, 95% CI: 1.02, 1.24) as well as amphetamines (IRR=1.18; 95% CI: 1.06, 1.33) however this increase was not observed in other road user groups. The prevalence of ketamine increased in motor vehicle drivers (IRR=1.38, 95% CI: 1.22, 1.57), motorcyclists (1.80, 95% CI: 1.07, 3.03) and pedestrians (IRR=1.29; 95% CI: 1.07, 1.55), but not in pedal cyclists. There were no changes over time in the prevalence of THC, benzodiazepines, antidepressants or antipsychotics.

Discussion
A decline in the prevalence of alcohol in pedestrians and motor vehicle drivers was observed. This suggests that law enforcement and public health strategies in Australia to address the road toll and drink-driving is having a positive effect on Victorian roads. However, increases were observed in the prevalence of opioids, amphetamines and ketamine in motorcyclists, which is consistent with the increasing use of these substances in the Australian population. It was noteworthy that the prevalence of these drug types was not observed in motor vehicle drivers, indicating that motorcyclists may present a higher risk group for drug-related road crashes. Cannabis use in Australia has remained steady which corresponds to the findings in this study. While illicit use of pharmaceuticals has increased in Australia in recent years, this was not reflected in the prevalence of benzodiazepines, antidepressants or antipsychotics in this study, supporting research that indicates these drugs have little impairment effect on drivers.

Conclusions
These data provide important insights into changes over time in the prevalence of alcohol and other drugs in road traffic crashes. These findings can be used to inform testing regimes and to develop targeted interventions to reduce alcohol and other drug use in all road users.
Abstract ID 363
When cultural differences breed a separate toxicological ecosystem: the province of Québec (Canada) and the opioids crisis.

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Aim
The opioids crisis has been developing for several years now on the North American continent, showing no signs of slowing down. In Canada, the opioids crisis unfurled from West to East, starting with the province of British Columbia and making its way towards the province of Québec and Maritime Provinces. For the first time in decades, the average life expectancy in British Columbia has decreased, due to a surge in opioids related deaths.

Nonetheless, partial data released by the Chief Public Health Officer of Canada in October 2018 seemed to indicate that the province of Québec was relatively spared by the phenomenon. To check whether the postmortem casework treated at the Laboratoire de sciences judiciaires et de médecine légale corroborate this assessment, a retrospective study of cases involving opioids was performed.

Methods
All postmortem cases for which at least one traditional or novel synthetic opioid was detected between January 2013 and December 2018 were included in this retrospective analysis. The date and location (postal code) of the event, age and sex of the deceased, cause of death determined by the pathologist and complete toxicological results in all biological matrices including concentrations where available, were recorded. R (programming environment) and RStudio (graphical interface, Boston, Massachusetts, USA) were used to manipulate the database and extract descriptive statistics.

Results
A total number of 472 postmortem cases where at least one opioid was detected were identified, out of a total of 4 023 postmortem cases treated over the period covered. The percentage of cases involving opioids showed no upwards or downwards trend, varying between 10% and 15%. The deceased were predominantly males (63%). Older individuals were overrepresented with respect to the general population, with deceased between 0 and 19 years old constituting 1% of cases, 20 to 29 years old 11%, 30 to 39 years old 19%, 40 to 49 years old 21%, 50 to 59 years old 22%, 60 to 69 years old 15% and 70 years and older 10%.

The seven most prevalent opioids were hydromorphone (31% of opioid cases), morphine (25%), fentanyl (17%), oxycodone (17%), codeine (11%), methadone (10%) and heroin (6-monoacetylmorphine) (6%). No upwards or downward trends in prevalence could be evidenced with passing years. More than one opioid could be observed in 40% of opioid cases. All cases combined several toxicologically relevant compounds, with acetylamphetamine, cocaine, amitriptyline and clonazepam being the substances most detected in combination with opioids.

Novel synthetic opioids (NSO) were detected in 11 cases over the studied period, more specifically acetylfentanyl, furanylfentanyl and U-47700. Their prevalence seemed to increase over time, although the low number of cases makes it difficult to draw a definite conclusion.

Discussion
No evidence of the tell-tale increase in fentanyl prevalence over time could be observed in the gathered data, nor an increase in the absolute or relative number of postmortem cases involving at least one opioid. This seems to confirm the data published by the Chief Public Health Officer of Canada, and indicate that the opioids crisis has not materialized in the province of Québec, Canada.

However, toxicology analyses are not performed systematically postmortem, and before November 2018, part of the postmortem toxicology workload of a non-judicial nature was carried out by a different laboratory whose statistics are not included in the present study. This of course limits the inferences which can be drawn from the database.

Québec is the only province in North America whose official language is French. This relative cultural isolation seems to have an impact on the toxicological ecosystem observed. It was already known that although the number of opioids prescription per capita was similar to other provinces, the defined daily dose was the lowest of all provinces. Additionally, statistics for driving under the influence of drugs (DUID) cases have shown for several years that stimulants (methamphetamine, cocaine) were more popular than opioids and benzodiazepines in Québec, a trend opposite to what was observed in the neighbouring province of Ontario. It isn't the first time a “toxicological ecosystem” of the sort is observed in the province. Indeed, GHB was detected on average in 24% of all DUID cases analyzed over the 2013-2018 period. In the neighbouring province of Ontario, this number drops to less than 1%.

Conclusions
Statistics of opioids detected in postmortem casework in Québec (Canada) seems to confirm the preliminary published data suggesting that the opioids crisis has not taken hold in this province, making it Astérix’s small village of indomitable Gauls holding out against the opioids invader. Further data compilation and analysis in the upcoming years will allow to keep an accurate portrait and pinpoint the emergence of specific trends amongst postmortem casework.
Abstract ID 368
Research on the generation of Ethanol non-oxidation metabolites in various human blood samples.
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Aim
EtG (ethyl glucuronide) and EtS (ethyl sulfate), the primary non-oxidation metabolites of alcohol in body fluid or keratin, were widely used as biomarkers to determine alcohol consumption. When the biological sample decayed, ethanol would generate because of fermentation of microbe. Since some paper reported that E.coli could accelerate the decomposition of EtG, there were opinions that supposed ethanol in urine might be metabolized into EtG or EtS, by microbe which caused urinary tract infection, as well as in blood. This might result in false positive, but remained unproven. In forensic toxicology, false negative and positive are significant issues that should be avoided. Especially in recent years, drunk driving was determined as crime. To ensure the accuracy of forensic-analysis results, this study aimed at assessing the application of EtG and EtS to distinguish the source of alcohol in blood. The generation of EtG and EtS was studied in blood samples from various individuals, to assess if they could be ideal biomarkers of before-death alcohol consumption.

Methods
To detect Ethanol in blood samples, Headspace-Gas chromatography was applied according to the national standard (GAT1073-2013). SPE (Solid Phase Extraction) and LC-MS/MS method were optimized to analyze EtG, EtS. For extraction, NH2 cartridges were conditioned by 2mL MeOH (0.3% Formic Acid, FA) and 2mL H2O. After blood samples were loaded, 2mL MeOH (0.3% FA) was added to wash. Elution was carried on by 2mL MeOH (5% NH3·H2O). The eluent was dried in 35°C water bath under nitrogen stream and the residue was dissolve by 10% ACN. Separation of EtG and EtS was performed by SB-C18 column, using H2O-ACN (9:1) as mobile phase. Four group samples were prepared: blank venous blood, negative post-mortem blood, spiked venous blood samples with blood alcohol concentration (BAC) at level A (80mg/mL) and level B (20mg/mL). There were 6 samples in each group. Every sample was divided into 5 aliquots and then kept in 5 kinds of conditions: ambient temperature(AT), AT with NaF addition, 4°C, 4°C with NaF addition and -20°C. EtOH, EtS and EtG blood concentrations were monitored during 2-month storage.

Results
According to the results of method validation (U.S.FDA), LLOQ of EtG and EtS were lowered to 0.025μg/mL and 0.040μg/mL respectively, with the recovery ranged from 85-115%. Both the precision and accuracy of developed method met the criteria. There was good linearity in range of 0.050-2.5μg/mL(R2>0.999). As for the results of analysis, during monitoring period, five of six post-mortem(PM) samples were detected EtOH positive after being kept in AT for only 2 days, with BAC of three of them up to 50.60mg/mL, 51.96mg/100mL and 64.27mg/100mL respectively. Those PM samples kept in 4°C were detected EtOH positive 3 days later with the highest BAC up to 17.76mg/100mL (legal limit in China: 20mg/100mL). All the blank venous samples were EtOH negative during the whole monitor period. As for the spiked samples, EtOH concentration of AT samples decreased to be lower than LLOD after 2 months and refrigeration samples were more stable. It was worth noting that all the samples were EtG and EtS negative through the whole period.

Discussion
The developed method was validated to be with better sensitivity and recovery, while LLOQs of the deproteinization method for serum established by Luca Morini etc. (Ethyl glucuronide and ethyl sulphate determination in serum by liquid chromatography–electrospray tandem mass spectrometry, Clinica Chimica Acta, 2007) were 40ng/mL and 50ng/mL respectively and both LLOQs of the method established by Hui Zhao were 50ng/mL (Determination of Ethyl Glucuronide in Blood and Urine by LC-MS/MS, Journal of Forensic Medicine, in Chinese, 2010). Compared to generation of EtOH within 3 days in post-mortem blood stored in AT, no alcohol generation was detected in venous blood. However, unlike the common cognition in forensic, the peak BACs of several PM samples at AT were much higher than the legal limit. It meant that the only detection of EtOH would probably lead to false positive for PM samples.

Conclusions
According to results obtained so far, EtOH generated due to decay could not be metabolized into detectable amount of EtG and EtS, neither did artificially additional EtOH. Thus, EtG and EtS were validated effective as specific biomarkers of before–death consumption of alcohol. It would help avoiding false-positive results of alcohol-consumption determination to analyze EtG and EtS in PM blood samples.
Abstract ID 371
Detection of poisonous phosphine gas in exhumed specimen.
Sohail Shahzad
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PFSA

Aim
Aluminum and zinc phosphides are commonly used as insecticides and rodenticides respectively. These are very cheap, easily available and highly toxic compounds therefore commonly used as suicidal or homicidal poisons. Toxic effects of these phosphides are due to liberation of phosphine gas. Generally it is believed that phosphine would not be retained by exhumation specimens and escape during decomposition processes of cadaver. Therefore, chances for its detection in exhumed specimens are limited. Here we report a case in which phosphine was detected in decomposed and putrefied stomach contents of a female six months after her death and burial.

Methods
Presumptive testing was performed using silver nitrate colorimetric test in Conway micro diffusion cell followed by confirmation with Gas chromatography nitrogen phosphorous detector technique (GC-NPD) using PLOT column.

Results
Silver nitrate impregnated strips turned black which indicated the presence of phosphine. Presence of phosphine was confirmed on GC-NPD on the basis of retention time which was similar to positive control.

Discussion
Exhumation specimens are a real challenge for analysts to detect poisonous gases especially when time between burial and postmortem is in several months. It was observed that stomach contents had paste like consistency which might have resulted in retention of intact phosphide due to unavailability of water for reaction.

Conclusions
It is a first case of phosphine detection in exhumation specimens as per our knowledge.

Abstract ID 372
A dothiepin related death with unusually high tissue concentration.
Sohail Shahzad
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PFSA

Aim
Dothiepin (dosulepin) is a tricyclic antidepressant (TCA) drug, used for treatment of depression. Currently it is not being widely used due to its high toxicity and availability of relatively safer antidepressants. A man previously in good health condition, with no previous history of depression or use of antidepressant drugs, was found in comatose in periphery of Pakistan. He was admitted to nearby hospital but could not survive. As it was a matter of suspected death, autopsy was performed and deceased's stomach contents and liver samples were sent for toxicological analysis. The main objective of this work is to publish lethal aspects of dothiepin and its abuse for homicidal or suicidal killing.

Methods
Samples including stomach contents and liver were extracted for drugs under alkaline conditions and run on GCMS in scan mode using DB-5MS column. Confirmation and quantitation for dothiepin was performed using ion of m/z 58.1 as quantifier, while ions of m/z 202.1 and 203.1 were used as qualifiers. Trazodone was used as internal standard by monitoring its ions of m/z 70.1 and 176.1.

Results
During drug screen analysis, dothiepin was detected in stomach contents and liver of deceased. Peak areas in both specimens were very high indicating intake of huge amount of dothiepin. Quantitation has revealed lethal concentration of dothiepin (greater than 104 mg/Kg) in liver and stomach contents of deceased. Undigested pink colored tablets of dothiepin were also present in stomach contents.

Discussion
Dothiepin is a potent antidepressant drug with therapeutic blood concentrations range from 0.003-0.12 mg/l. Its toxicity starts when blood concentration go over 0.8 mg/l. Reported lethal concentration of dothiepin in liver is ranged from 2-52 mg/kg. In current study liver and stomach concentrations which were at least twice lethal than that was reported in literature.

Conclusions
These findings strongly suggested the acute exposure of higher doses of dothiepen to the deceased before death. Thus, it was confirmed that dothiepin was used in suicidal or homicidal killing of a young man.
Abstract ID 378
Streamlining Unknown Screening for Postmortem Analysis.
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Aim
Accurate identification of drugs present in postmortem samples is critical for forensic toxicologists to successfully carry a case examination. The use of high resolution mass spectrometry (MS) in the forensic laboratory enables toxicologists to rapidly obtain complete chemical profiles from biological samples, which subsequently leads to increased confidence in compound identification through accurate mass information at low analyte concentration.

Here, a comprehensive drug screening workflow for the analysis of postmortem blood samples is described. The workflow enabled confident unknown substance identification within an efficient, all-in-one workflow. This comprehensive drug screening workflow enabled reliable compound fragmentation comparison to library spectra for confident drug identification and retrospective analysis to avoid missing potential drugs present in postmortem samples.

Methods
Control whole blood samples were spiked with a stock standard solution mixture and extracted for LC/MS screening to determine the retention times. Forensic case postmortem blood samples were extracted by using a protein precipitation procedure followed by centrifugation; supernatant was evaporated and reconstituted in mobile phase for analysis. Analytes were chromatographically separated at 30˚C using a Phenomenex Kinetex phenyl-hexyl (50 x 2.1 mm, 2.6 µm) column. Mobile phases were water and methanol with appropriate additives, 1 mL/min flow rate. Mass spectrometric detection was conducted on a X500R QTOF System operated in positive electrospray mode using SWATH® Acquisition. SWATH Acquisition enabled to collect MS and MS/MS information on every detectable peak within a sample, essentially creating a digital record of each sample analyzed. Samples were evaluated against four main confidence criteria weighted as follows: mass error (15%), retention time (30%), isotope ratio difference (5%), and library score (50%) for all compounds. These criteria were used to generate a combined score. The processing criteria for positive identification of an analyte in a sample required all four main confidence criteria to pass.

Results
The spiked control whole blood samples were successfully used to obtain retention times and MS/MS quality spectra to build a data analysis processing method and high resolution spectral libraries for the 153 target analytes. SWATH Acquisition was employed to obtain unrestricted fragment ion spectra generation over the whole run, minimizing the risk of missing potential forensic compounds present in postmortem samples. This detection mode was found beneficial as it generated comprehensive and high quality MS/MS spectra, enabling reliable compound fragmentation for spectra library database searching for most of the analytes. In addition, this detection strategy provided the option to comprehensively re-interrogate the sample data should new questions arise in the future. For example, the investigation of a potential known substance (i.e., Acetyl Fentanyl) present in the sample was explored by retrospectively mining the data processing method with the compound’s molecular formula (C21H26N2O).

Discussion
The implementation of a robust method development process resulted in high combined scores for all compounds based on the four main confidence criteria defined in the processing method. These criteria were automatically calculated for all the forensic compounds detected in postmortem samples and visualized using a traffic light pattern on the software, such that compounds identified with high confidence are indicated using green check symbols. These traffic lights can be sorted and filtered by identification criteria for review and used to positively report identified compounds. Additional quantification were implemented by setting an analyte concentration threshold based on the LODs to minimize false positives and/or false negative hits, which resulted sub ng/mL LOD for the majority of the drugs screened.

Conclusions
Overall, the developed QTOF-MS screening approach enabled the rapid implementation and optimization of the screening workflow for 159 compounds of interest for confident drug identification and retrospective analysis to avoid missing potential drugs present in postmortem samples.

Orthodoxia Mastrogianni

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Aim

Almost 500 000 people are annually estimated to die around the world as a result of drowning, the second leading cause of injury related deaths after traffic accidents. The last few decades in Greece, drowning in water consists one of the leading causes of unintentional injury deaths. More specifically, in the northern part of the country (Thessaly, Macedonia), a coastal area and a very popular summer destination in the Balkan region which attracts thousands of tourists each year, the number of deaths related to drowning consistently increases. The aim of this study is to determine the true burden of drowning in this region and to further evaluate the relationship between the occurrence of fatal drowning incidents in this region and the demographic characteristics, health history of the deceased and the presence of psychoactive drugs in blood and urine respectively.

Methods

Post mortem blood and urine samples were collected and analyzed for forensic purposes. A preliminary immunoassay was performed in urine for cannabinoids, cocaine, amphetamine, opiates and benzodiazepines. Alcohol determination was done using Head Space-Gas Chromatography with Flame Ionization Detector. Gas Chromatography-Mass Spectrometry (GC-MS) was used as confirmatory method in order to determine the drugs levels in blood and urine after liquid-liquid extraction (LLE) for sample preparation.

Active substances were coded and categorized on the basis of the Anatomical Therapeutic Chemical (ATC) classification system, and an extension made for drugs of abuse without a valid ATC classification. The forensic autopsy findings were acquired from the medical examiners' requests for toxicological analysis and police reports and were coded according to the June 2018 release version of the International Classification of Diseases (ICD-11). The location of the incident was grouped according to the European Union's Nomenclature of Territorial Units for Statistics (NUTS).

Results

A total of 324 fatal drowning were on record to have occurred in the Greek statistical regions of Northern Greece and Thessaly during the period between 2009 and 2018 and the majority of them (98.1%) in saltwater. The victims were predominantly male (69.3%) with a mean age (± standard deviation) of 68.86 (± 14.5) years old. The majority of the deaths were recorded in the region of Central Macedonia (87.3%) and especially in the peninsula of Chalkidiki (45.5%), Pieria (22.0%), and Thessaloniki (19.5%). While most of the victims were Greeks (67.4%), a notable portion of them were foreign nationals (32.6%). Blood alcohol analysis was performed to 223 of the victims and among these, a percentage of 41.7% had consumed alcohol, whilst only a portion of 15.7% was above the limit of 0.2 g/L. Toxicological analysis was performed only to 184 of these cases, since from 2009 to 2014, prosecutors ordered only a forensic pathology examination without the conduction of toxicological analysis for some of these incidents.

Discussion

The substances detected with the highest frequency were antidepressants (ATC: N06A, 38.8%), particularly SSRIs (ATC: N06AB, 23.5%), as well as anxiolytics (ATC: N05B, 18.4%), particularly anxiolytic benzodiazepine derivatives (ATC: N05BA, 17.3%). Preliminary data shows that anxiolytics were found across age groups and at similar frequencies across gender, but antidepressants were more prevalent in elderly and middle-aged victims (the statistical data is still in process). The post-mortem findings largely pertained to circulatory system comorbidities (90.1%), such as ischaemic heart diseases (ICD-11: BA40-BA60, 37.3%) and diseases of the coronary artery (ICD-11: BA80-BA86, 26.9%) and were mainly found in the elderly (>65 y.o.).

Conclusions

The presented data indicate that older males, and Greeks were mainly involved in fatal drownings. Cardiovascular issues, which are common in older people are likely to have been involved in the negative outcome. Antidepressants and anxiolytics drugs coincided with a plurality of fatalities and maybe are an overlooked risk factor in drowning due to their effects on psychomotor function and cognition.
Abstract ID 383
Development of GC/MS method for THC determination in blood and application in real samples from dead drivers in Greece.
Orthodoxia Mastrogianni
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Aim
In Greece, the law for driving under the influence of drugs is very general without adopting a zero-tolerance approach or establishing level limits of drugs in blood indicating impairment. According to the “unclear” Greek legislation on DUID, it is not obligatory to determine the drug level in blood. Despite this fact our lab proceeds to the quantification of Δ9-THC in order to confirm the influence of cannabis at the driving ability. The aim of this study was the development and validation of a GC-MS method for the determination of THC in blood and finally its application in blood samples collected from drivers involved in traffic accidents.

Methods
Blood samples were collected from drivers involved in fatal traffic incidents.

Analysis of THC was performed on a gas chromatograph Agilent Technologies 7890A with a MS 5975C inrXL, EI/CI MSD with Triple-Axis detector, after liquid-liquid extraction. Five different organic solvents (Butyl Acetate, Di-isopropyl ether, iso-octane, n-hexane and n-heptane) were used for the optimization of the extraction procedure. Two different methods were developed for the determination of THC in blood without derivatization of the analyte and derivatization with BSTFA+ 1% TMCS.

Results
The quantification of THC was done by selective ion monitoring (SIM) mode, using 386 as the quantifier ion for the THC-TMS derivative and 299 for the THC respectively. Hexane was the solvent of choice for the extraction procedure. Both methods were linear in the tested range 2-80 ng/mL (R2:0.9976 without derivatization and 0.9995 for the method with the derivatisation step, respectively). Both methods gave comparable accuracy and precision values. Finally, in terms of LOD (limit of Detection) and LOQ (limit of Quantitation), the method with the derivatisation step showed better results (LOD: 0.3ng/mL versus 0.6 ng/mL, LOQ: 1.0 ng/mL versus 2.0 ng/mL) and thus, this validated protocol was applied to real post-mortem blood samples from drivers involved in fatal accidents. Finally, we analyzed 270 blood samples and 15 of them (5.6%) were positive for cannabis. The THC concentrations were ranged between 3.6 -200 ng/mL.

Discussion
Finally, in terms of LOD (limit of Detection) and LOQ (limit of Quantitation), the method with the derivatisation step showed better results (LOD: 0.3ng/mL versus 0.6 ng/mL, LOQ: 1.0 ng/mL versus 2.0 ng/mL) and thus, this validated protocol was applied to real post-mortem blood samples from drivers involved in fatal accidents. Finally, we analyzed 270 blood samples and 15 of them (5.6%) were positive for cannabis. The THC concentrations were ranged between 3.6 -200 ng/mL.

Conclusions
A rapid, sensitive and reliable method was developed for the determination of THC in blood after optimization experiments and finally successfully applied to 270 real blood samples from dead drivers involved in traffic accidents.
Abstract ID  394
Death by drowning after consumption of high dose of methadone: A case report.
Cláudia Margalho
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Aim
Methadone (MTD) is a synthetic opioid agonist, used especially in detoxification and maintenance programs, to reduce opioid cravings and prevent abstinence symptoms. It can also be used as a sedative, analgesic or recreational drug (purchased in the black market). After administration, MTD is mainly metabolized in its metabolite 2-ethylidine-1,5dimethyl-3,3-diphenylpyrrolidine (EDDP). According to the European report on Drugs (2018), MTD is often used improperly. However, it is the most prescribed opiate in substitution treatments, administered to 63% of users (in Portugal this percentage rounds 70%). The MTD maintenance treatment can be initiated in maintenance therapy centres and it is free of charge to the user. According to the National Institute of Legal Medicine and Forensic Sciences (INMLCF, I.P), eventhough MTD was detected in 42% of deaths by overdose, it was only detected in 13% of cases related with homicide, suicide, natural death or accidents. Methadone intoxication can occur accidentally (either by substance abuse, mainly amongst addicts, or children consumption) or intentionally for suicide or homicide purposes. This work describes one case of death by drowning where an unsuspected high concentration of MTD in peripheral blood was found. A 45-year old, HIV-infected, drug addict male individual was found floating offshore after 5 days of disappearance.

Methods
Peripheral blood collected during the autopsy exhibited high fluidity. This was the unique matrix sent to the forensic toxicology laboratory to perform all the required analyzes. Drugs of abuse and benzodiazepines were firstly analysed by immunoassay methodology and the screening of ethanol was made by gas chromatography-flame ionization detector with headspace injection (GC-FID-HS). The systematic toxicological analyses were performed by gas chromatography-mass spectrometry (GC-MS), for drugs of abuse and unknown substances, and by ultra-high-performance liquid chromatography-mass spectrometry (UPLC-MS-MS) for prescription drugs, after solid-phase extraction.

Results
Detection and quantification of MTD and EDDP (4539.0 ng/mL and 10.02 ng/mL, respectively) was performed in blood by GC-MS. The immunoassay results were positive for benzodiazepines and negative for drugs of abuse. The blood analysis, performed by UPLC-MS-MS, was positive for alprazolam (12.0 ng/mL), n-desalkylflurazepam (18.0 ng/mL) and midazolam (66.0 ng/mL). Ethanol and 1-butanol were found in blood through GC-FID-HS with concentrations of 0.77 g/L and 0.43 g/L, respectively.

Discussion
Some authors refer the existence of a close biological and behavioral connection between HIV seropositivity and a high risk of overdose. Several published studies have demonstrated that methadone presents variable postmortem redistribution from individual to individual. However, recent works have shown that methadone concentration changes in peripheral blood were considered irrelevant in forensic cases interpretation. The high concentration of methadone compared to its metabolite EDDP, suggests recent ingestion prior to death. The ethanol and 1-butanol detection suggests postmortem microbial production.

Conclusions
This case emphasizes the difficulties and limitations, faced by the forensic toxicologists, in interpreting toxicological results in putrefactive matrices and with scarce information available. Based on circumstantial evidence, autopsy findings and results from the toxicological analyses, it is possible to conclude that combined effects of high concentration of methadone, in addition to alprazolam and midazolam found in postmortem peripheral blood, could have contributed to the individual's death.
Abstract ID 421
Fentanyl abuse in Greece: Presentation of two case reports of fatal intoxication as a result of illegal recreational use with accompanying genetic data.
Orthodoxia Mastrogianni
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Aim
Illegal use of fentanyl is on the rise in Europe, raising concerns about an upward trend of abuse and accompanying mortality similar to the one established in the USA. Risk of death is increased by the simultaneous consumption of other controlled substances or CNS drugs, and genetic predisposition to increased biological response may also play a part. In Greece, there is so far little recorded evidence of synthetic opioid abuse. Here we report two different cases of fatal fentanyl intoxication with ostensibly different characteristics.

Methods
For the first case, buprenorphine and fentanyl were determined by UPLC-MS/MS analysis following simple and efficient sample pretreatment. The method showed dynamic linear range from 0.5 to 6000 ng/mL with linearity expressed by regression coefficient (R2) fentanyl (0.9980) and buprenorphine (0.9988). The method of analysis was quick with 17 min total run time and analytes were eluted at 8.96 min (Fentanyl) and at 9.16 min (buprenorphine). THC-COOH was determined in the blood of the deceased by Gas Chromatography – Mass spectrometry Analysis (GC/MS) after Liquid –Liquid Extraction. (LLE)
Liquid-liquid extraction (LLE) was also used for the isolation of the drugs from blood and urine samples in case 2 with subsequent GC/MS Analysis. The calibration curve for fentanyl showed linearity in the range 10-500 ng/ml, with R2 coefficient 0.9965.

Genotyping for the pharmacodynamically important OPRM1 A118G (rs1799971), COMT Val158Met (rs4680), and SLC6A4 triallelic 5’HTTLPR (rs4795541; rs25531) polymorphisms, as well as the ABCB1 3435C>T (rs1045642) polymorphisms was accomplished with previously published RFLP-PCR/PCR methods.

Results
Case 1: On December 2017, a 16 year old male collapsed at his home, in the city of Volos (central Greece), after having spent the previous night out with his friends. The deceased was taken to the hospital where resuscitation attempts at the ICU unit were unsuccessful. A bag containing a white powder later identified as buprenorphine was found in his clothes. Toxicological Analysis of femoral blood revealed the presence of 0.60 ng/mL fentanyl, 0.94 ng/mL buprenorphine and Tetrahydrocannabinolic acid (THC-COOH).

Case 2: On November 2018, a 37 year old male was found dead at his home, in the northern Greek city of Kozani. The deceased had a history of drug abuse and the immediate cause of death was attributed to pulmonary edema. Toxicological analysis of femoral blood showed 20.0 ng/mL fentanyl, 7-amino flunitrazepam(7-AF), diazepam, nordiazepam, THC-COOH; urine: fentanyl, 7-AF, lorazepam, and THC-COOH. Both cases were homozygotes for the OPRM1 118A (major) allele, and heterozygotes for the COMT polymorphism (Val/Met). Case 1 was of the low 5’HTT expressing (SLC6A4 SS), whereas case 2 of the high 5’HTT expressing (SLC6A4 LALA) type. Case 2 was homozygous for the ABCB1 3435T allele (TT); case 1 was a heterozygote for ABCB1 3435 (CT).

Discussion
Genotyping was performed as part of larger effort to discover genetic factors associated with propensity for abuse, and sensitivity to fentanyl and new synthetic opioids. All four genes examined in this report were associated in the past with response to pain and opioid requirements; the OPRM1 118G allele has been associated with drug addiction and the 5’HTTLPR SS genotype with higher risk for substance use disorders and with inefficient addiction treatment. The COMT 158Met allele has been associated with high sensitivity, and the ABCB1 3435TT genotype with high exposure to opioids. In this report, the 5’HTTLPR SS genotype may have contributed to the fatal intoxication of case 1, and the ABCB1 3435TT to that of case 2, but a lot more cases need to be examined in order to reach a safe conclusion.

Conclusions
In both cases, the proximal cause of death was fentanyl intoxication possibly complicated by pharmacodynamic interactions with buprenorphine (case 1) or diazepam (case 2). It is too early to assess the significance of genetic predisposition.

Acknowledgment: Financial support was obtained from a Project from the European Commission under the Call JUST-2017-AG-DRUG SUPPORTING INITIATIVES IN THE FIELD OF DRUGS POLICY Topic: JUST-2017-AG-DRUG; Type of action: JUST-DRUGS-AG (GRANT AGREEMENT NUMBER: 806996-JUSTSO)
Abstract ID 423
Comparison of endogenous blood, urine and vitreous humour gamma-hydroxybutyric (GHB) post-mortem concentrations with those from fatal and non-fatal intoxication.
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Aim
This study aims to compare the blood, urine and vitreous humour GHB concentrations from GHB-related deaths (i.e. overdose attributed to GHB or GBL toxicity) with those from non GHB-related deaths (i.e. where death is attributed to an unrelated cause such as a natural death, violent death, trauma or suicide). A further comparison is made with blood GHB concentrations from cases of non-fatal GHB/GBL intoxication.

Methods
Where available, at least two samples (generally either blood and urine or blood and vitreous humour) from each individual were analysed for GHB. For blood and vitreous humour, the samples were analysed by gas-chromatography-mass spectrometry (GC-MS) after liquid-liquid extraction and derivitisation. For urine, the samples were analysed by liquid-chromatography-mass spectrometry (LC-MS). The analysis covers a calibration range of 10 to 200 mg/L for blood or vitreous humour and 3 to 150 mg/L for urine, with samples being diluted where necessary.

Results
This study presents the blood, urine and vitreous humour GHB concentrations from a large number of post-mortem cases analysed at the Toxicology Department of the Teddington Laboratory, United Kingdom, over an approximate two-year period from 2017 to 2019. For comparison, the results are split into two groups: GHB-related deaths (i.e. overdose attributed to GHB or GBL toxicity) and non GHB-related deaths (i.e. where death is attributed to an unrelated cause such as a natural death, violent death, trauma or suicide). The blood GHB concentrations from a separate group of cases involving non-fatal GHB/GBL intoxication are included for further comparison.

Discussion
Gamma-hydroxybutyrate (GHB) and gammabutyrolactone (GBL) are relatively common drugs of abuse. They are of particular interest in fatal cases involving abuse of these drugs, suspected administration in drug facilitated crime (DFC) and driving under the influence of drugs. However, the now well-known presence of GHB as a natural substance in human tissues and particularly post-mortem production causes interpretation issues.

With respect to post-mortem samples, the data from this study confirms the usefulness of analysing an additional sample for GHB other than blood, with urine being particularly useful. The majority of blood GHB concentrations in the non GHB-related deaths were low (generally less than 30 mg/L), with low or negative urine GHB concentrations providing further support for the results from the blood sample being from GHB production. Vitreous humour GHB concentrations were generally found to be very similar to the corresponding blood concentrations.

The blood concentrations from both fatal cases attributed to GHB/GBL toxicity and non-fatal GHB/GBL intoxication were highly variable.

Conclusions
The data from this study confirms the usefulness of analysing an additional post-mortem sample for GHB other than blood, with urine being particularly useful. Analysis of vitreous humour can be useful when urine is not available, although vitreous humour GHB concentrations were generally similar to the corresponding blood concentrations.
Abstract ID 427
A comparative study of ethyl alcohol concentration in coastal cartilage in relation to blood and urine.
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Aim
In some forensic autopsies blood is not available hence other matrices are sampled for toxicological analysis. The aims of the present study were: to examine whether ethyl alcohol can be detected in costal cartilage and to investigate whether analyses of different forms of costal cartilage can give useful information about ethyl alcohol concentrations in peripheral blood. This paper presents the results of a comparative study of ethyl alcohol concentration in post-mortem costal cartilage (CC), blood, and urine samples collected during medico-legal autopsies. Examination of this type of material may be useful in cases: 1) when soft tissues are not available or in a state of advanced cadaver decomposition (as an alternative material), 2) when the remains are almost completely skeletonized (as the main material).

Methods
Ethanol concentrations were determined in samples of unground costal cartilage (UCC), ground costal cartilage (GCC), femoral venous blood (FVB), and urine (U). UCC was obtained by scalpel fragmentation whereas GCC - by grinding in Cryogenic Mill 6770 SpexSamplePrep (3 min preincubation, 1 grinding cycle 2 min, 12 CPS). The studied group included CCs taken from cadavers in which the presence of ethyl alcohol in blood and urine was demonstrated. The control group consisted of CCs taken from cadavers with no ethyl alcohol detected. The samples were analysed in duplicate by gas chromatography (GC) with a flame ionization detector (FID) using the headspace analysis. The chromatographic separation was performed with a Restek BAC2 column. T-butyl alcohol was used as an internal standard. Results were obtained for 100 samples. Eight-point calibration curve for ethyl alcohol in mg/ml or mg/g (0; 0.1; 0.2; 0.5; 0.8; 1; 2; 3) was linear in the whole range. The limit of detection (LOD) was determined as 0.05 mg/ml or mg/g and limit of quantification (LOQ) as 0.1 mg/ml or mg/g. All measurements were carried out in a certified laboratory (GTFCH, Proficiency test – ETOH 2/19).

Results
The statistical analysis indicated a strong positive correlation between alcohol levels in UCC and GCC methods (r = 0.734, p < 0.001). Additionally, the concentrations of GCC and UCC ethanol and femoral blood were compared. Based on the constructed ROC curves, the optimal cut-off point determining the sobriety (alcohol content in the cartilage) was 0.235 mg/g for the UCC method and 0.168 mg/g for the GCC method, respectively. To measure the accuracy of the diagnostic tests (UCC and GCC), the area under a ROC curve (AUC) was analysed. In all cases, we interpreted AUC value as excellent diagnostic accuracy (AUGUCC = 0.897; AUGGCC = 0.984).

Discussion
The study showed that higher ethanol concentrations were determined in ground samples. The ethanol distribution ratios GCS/FVB show wide variation and this calls for caution when results of post-mortem costal cartilage ethanol concentration are used to estimate its concentration in FVB.

Conclusions
According to our best knowledge, we are the first team which demonstrated the possibility of ethyl alcohol detection in the post-mortem costal cartilage.
Abstract ID 434

Quantitative analysis of nitrites and nitrates in biological specimens from the suicidal cases by ingestion of sodium nitrite using ion chromatography.

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Aim
Sodium Nitrite (NaNO₂) is a white to slightly yellowish crystalline powder. It is used as a food additive in processed meat and fish product as it could inhibit microbial growth and develop a desirable red color of meat. In chemical industry, the main use of sodium nitrite is for diazo or nitroso compounds production, and it also used as an anti-freezing agent in the concrete by lowering the freezing point of the water. As nitrite has vasodilation effect, it can be used as a vasodilator in angina pectoris or arteriosclerosis. Nitrite is a potent oxidizing agent and oxidation of hemoglobin by nitrite may result in methemoglobinemia. Headache, skin flushing, nausea, vomit, diarrhea and orthostatic hypotension with reflex tachycardia are the most common adverse effects of nitrite, and nitrite intoxication could be fatal due to respiratory paralysis. Ingestion of as little as 15 mL of butyl nitrite can produce 40% methemoglobinemia in an adult, and the estimated adult lethal oral dose of sodium nitrite is 1 g. Recently, in South Korea, there have been frequent cases of suicide by purchasing sodium nitrite through the internet suicide site and taking it. In this study, we analyzed nitrite and nitrate in biological samples from six suicidal cases (aged 19–29, 5 male and 1 female) from January 2018 to April 2019.

Methods
Ion chromatography was used for the analysis of target anions. Biological samples were prepared using Amicon-Ultra centrifugal filter, and DionexIonPac TM AS9-HS column was used for separation. Mobile phase was 9 mM Na₂CO₃, and the flow rate was 1 mL/min.

Results
The concentration ranges of nitrites in heart blood, peripheral blood and gastric contents were 1.1 - 318.0 mg/L, 0.1-2.1 mg/L, and 300-59,230 mg/L, respectively, and the nitrates were 8.0-587.7 mg/L, 177.0-462.6 mg/L, and 962-3,100 mg/L, respectively.

Discussion

Conclusions
As nitrite is one of the most poisonous chemicals but it can’t be detected from routine toxicological screening such as GC-MS or LC-MS, it is highly recommended to check nitrite in methemoglobinemia.
Abstract ID 436
Determination of aluminium and zinc to opine cases of alleged phosphide poisoning using inductively coupled plasma-atomic emission spectrophotometry (ICP-AES).

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Aim
Quantitative estimation of the values of aluminium and zinc in alleged phosphide poisoning deaths to opine the cases conclusively.

Methods
A total of 110 such poisoning cases were collected from Mortuary All India Institute of Medical Sciences(AIIMS),New Delhi, State Forensic Science Laboratory (FSL), Delhi & State Forensic Science Laboratory, Haryana along with 100 control cases from Mortuary, AIIMS during the three year period (2014-16) after the ethical clearance obtained from the AIIMS,New Delhi ethics committee. Each case consisted of three biological samples i.e. Blood, Liver and Stomach contents. After screening all the samples were then digested using Microwave Digestion system and quantitatively analysed using Inductively Coupled Plasma-Atomic Emission Spectrophotometry (ICP-AES). A method was developed and validated followed by analysis of all biological samples.

Results
The values of aluminium obtained in blood, liver and stomach contents of alleged phosphide poisoning cases (n=27) were significantly higher than the control cases (n=100) samples. In blood the mean value was 32.87mg/l while in control cases it was 4.58mg/l. Similarly the mean value of zinc in liver and stomach contents of zinc phosphide poisoning cases were 51.10mg/g and 1383.01mg/l respectively while in control cases it was 5.95mg/g and 82.40mg/l respectively. All results were significant as p value <0.01 in all cases.

The values of zinc obtained in blood, liver and stomach contents of alleged phosphide poisoning cases (n=27) were significantly higher than the control case (n=100) samples. In blood the mean value was 40.23mg/l while in control case it was 14.21mg/l. Similarly the mean value of zinc in liver and stomach contents of zinc phosphide poisoning cases were 57.44mg/g and 44.44mg/l respectively while in control cases it was 25.66mg/g and 7.95mg/l respectively. All results were significant as p value <0.01 in all cases.

Discussion
The levels of aluminium were also present in the control case samples although it is not an essential part of human diet and body. According to the hypothesis aluminium levels were supposed to be found nil in the control cases samples. The probable reason for its presence in control cases which were normal humans is its consumption through various direct and indirect sources like antacids, utensils, packaged food items etc. The zinc levels found in control cases were highest in liver which is an indicator of long term zinc intake through food and various multivitamins. The results obtained in blood and stomach contents and after their comparison; it was found that in spite of the fact that zinc was an essential element of the body its presence in zinc phosphide poisoning cases was much higher than control cases.

Conclusions
Use of aluminium and zinc phosphide (AlP&ZnP) as an agricultural fumigant and rodenticide in India is massive because of agricultural sector. The considerable use, cheap and easy availability alongwith extremely high mortality enhances its misuse as a suicidal agent especially in northern part of country. Until now the cause of death used to be given on the basis of police inquest proceedings, post-mortem findings and presence of phosphine gas in viscera using a silver nitrate (AgNO3) test during toxicological examination. The case may be reported as false positive as putrefied viscera also give positive result and the cause of death remains inconclusive.

The present study not only estimates and compares the metal levels in poisoning versus control cases but it also gives an idea about the levels in normal deaths without the history of phosphide poisoning and which can also serves as a base for various further studies related to chronic exposure and toxicity related to these metals.
Abstract ID 557
Stomach contents as significant investigative tool in postmortem toxicological examinations.
Saima Afzal
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Aim
In postmortem cases, blood and urine are renowned as the most significant specimens to analyze drugs and their metabolites. However, in oral ingestion of drugs and poisons, stomach contents can provide important clues as high amounts of unabsorbed drugs or toxins may be detected. Objective of this study is to highlight the significance of stomach contents as specimen of interest in fatal oral ingestion in relevance to detection in post-mortem specimens of blood and liver.

Methods
In this study, authors considered data from four hundred and fifty cases submitted for toxicological examination on stomach contents, liver and blood specimens in their laboratory. These cases included screening for pesticides, drugs of abuse and other commonly ingested poisons and/or drugs. Poisons named cyanide, phosphine, para-phenylenediamine and its metabolites (diacetylated and monoacetylated para-phenylenediamine) were screened using colorimetric technique. Cyanide was confirmed by HS-GCFID, phosphine by GCNPD and para-phenylenediamine and its metabolites by GCMS. Drugs of abuse were screened using ELISA followed by GCMS confirmation. An additional broad range of drugs/pesticides was screened and confirmed by GCMS.

Results
One hundred and sixteen (25.7%) cases were found positive for the presence of drugs and/or poisons in stomach contents. Of these, 12.9% cases accounted for para-phenylenediamine and its metabolites, 14.6% for pesticides, 15.5% for phosphine and 1% for cyanide. Drugs of abuse in 17.2% and therapeutic drugs in 38.8% were detected, included benzodiazipines, opiates, amphetamines, ketamine, cannabinoids pheniramine, chlorpheniramine, metoclopramide, diphenhydramine, lidocaine, acetaminophen, atropine, laudanosine, clopidogrel, mefenamic acid, propranolol, ibuprofen, carbamazepine, mephenetermine, dextromethorphan, pyrimethamine, tramadol, chloroquine, nalbuphine, citalopram, verapamil, trimethoprim and orphenadrine.

Discussion
Stomach contents were positive for all para-phenylenediamine poisoning cases while only 47% said cases were positive in liver. Similarly, stomach contents were reported positive for all pesticide poisoning cases where as 59% of liver and blood stood negative. Phosphine was detected only in stomach contents. Drugs of abuse and therapeutic drugs were positive in all three specimens discussed. In poisoning cases, stomach contents have greater probability for detection of poisons depending on how much time elapsed between ingestion and death. In many cases of acute poisoning, undissolved capsules or tablets may be found in stomach contents, allowing quick drug or poison identification. Therefore, stomach contents are significant investigative tool in postmortem toxicological examinations.

Conclusions
Authors of this study recommend that testing on stomach contents can be a useful addition to poison testing protocol in postmortem cases especially for regions with high incidents of para-phenylenediamine (PPD) or pesticide exposure as many of poisons remain undetected in liver and blood which can produce false negative results.
Abstract ID 442
Medicolegal autopsies and toxicology analysis.
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Aim
The Algerian death investigation system is under the aegis of the public prosecutor, and he is sovereign to request a forensic autopsy or not. But for the complementary investigations that it is pathological or toxicological, they are under the responsibility of the legal doctor (there is no medical examiner or coroner system in Algeria).

The purpose of this study is quadruple: First, to determine the frequency of poisoning during medicolegal autopsies not only as a direct cause of death but also as a contributing factor; second to specify the route of introduction; third to investigate the existence of chronic anterior intoxication; forth to to know if the toxicological analysis should be systematic or not at all autopsies.

Methods
A retrospective survey covering three (03) years was conducted to evaluate the use of analytical toxicology at the time of forensic autopsy carried out in our forensic department of the University Hospital Center of Sidi Bel Abbes (Algeria).

The investigation is documented from medico-legal autopsy records conducted between January 1st, 2015 and December 31st, 2018.

No inclusion or exclusion criteria were considered, all autopsy records taken during this period were taken as a sample.

The main tests required are the search for ethyl alcohol, psychotropic substances, carbon monoxide and Toxic Chemicals in Agriculture.

Results
Among the 722 files analyzed, a request for toxicological analysis was found in 544 cases the presence of at least one xenobiotic was positive in 204 cases, xenobiotic identities were present in lethal concentrations in 25 cases. CO poisoning accounted for 15 cases, 05 cases for psychotropic drugs, 04 cases for Toxic Chemicals in Agriculture products and one case for misuse of household products. Alcohol was present in 44 cases with non-lethal doses (the cause of death was not toxic but violent).

Discussion
The contribution of toxicology is of paramount importance in forensics. This science, devoted to the study of poisons has made considerable progress with the appearance of new techniques.

Although in cases of violent death the autopsy most often finds the cause of death, this is not the case in the event of death where there is no evidence of violence, neither on the external examination nor at the autopsy. The same case arises in case of absence of pathological signs allowing to connect the death to his or that disease.

Conclusions
Medico-legal autopsy alone cannot find the cause of death in most cases. The toxicological analysis is of great use, on the one hand, to explain certain deaths, on the other hand, the cases where the toxic products are not the direct cause of the death but of the favorable factors, finally, the toxicological analysis must be systematic during forensic autopsies.

KEYWORDS: Toxicology; Autopsy; Cause of death; Retrospective study.
Abstract ID 446
Segmental analysis of aripiprazole in postmortem hair from psychiatric patients by use of liquid chromatography–tandem mass spectrometry.
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Aim
The present study investigated the antipsychotic drug aripiprazole and its metabolite dehydroaripiprazole in hair segments from deceased psychiatric patients in order to establish reference levels for aripiprazole and dehydroaripiprazole in hair. Assuming an average hair growth rate of 1 cm/month and depending on the length of the hair shafts, segmental hair analysis can provide information regarding drug exposure in months before death. Thus, by segmental hair analysis, patient compliance can be investigated. This is valuable information because overdoses or poor compliance related to the treatment with psychiatric drugs may be possible explanations for excess mortality recognized among psychiatric patients.

Methods
Hair samples were collected during autopsy from 15 individuals which were included in the autopsy-based study ‘SURVIVE’. The hair samples were collected from patients in which aripiprazole intake was known due to positive findings of aripiprazole in the respective postmortem blood samples and/or from patients with aripiprazole treatment mentioned in the case story. Among the 15 individuals two were assessed as having dyed hair, while the remaining had natural hair colour identified as brown (n=11), black (n=1) and grey (n=1). Quality control samples (QC’s) were prepared at three different concentration levels by soaking blank hair in a methanol:water (1:1, v/v) solution spiked with analytes. A method for extraction and analysis has previously been validated for single-dose studies (Wang et al.). This method was re-validated in order to include dehydroaripiprazole and examine an extended concentration range for both aripiprazole and dehydroaripiprazole by use of deuterated internal standards. Each hair sample was cut into 6 x 1 cm segments corresponding to the last six months prior to death, depending on the length of the hair shafts. The hair segments were decontaminated once with isopropanol and twice with water, then pulverized and extracted over night at 37°C in 300 µL extraction medium consisting of methanol, acetonitrile and 2 mM ammonium formate (25:29:46, v/v/v). After filtration 2 µL extract was injected into an UHPLC–MS/MS system (Wang et al.). The last aqueous wash fraction of each segment was diluted 1:1 and analyzed to test decontamination.

Results
The method was successfully revalidated in accordance with international guidelines and the validation parameters included linearity, matrix effect, recovery, precision, accuracy, lower limit of quantification (LLOQ), carry over and stability of extracts. The method was found to be linear in the concentration range from 0.005 (LLOQ) to 15 ng/mg for both aripiprazole and dehydroaripiprazole, with R2>0.99. CV was <15% at the studied concentrations of both analytes, while accuracy remained within 85–115% interval (75–125% at LLOQ for dehydroaripiprazole). Matrix effects were 73–88% (100% indicates zero matrix effect), while extraction recoveries ranged from 100–109% for the two analytes. The QC’s were analyzed during six runs and showed imprecision <13% within and between runs at each concentration level (n=13). The determined mean concentration for the QC’s were 0.068 ng/mg, 0.78 ng/mg and 2.3 ng/mg for aripiprazole and 0.082 ng/mg, 0.88 ng/mg and 2.8 ng/mg for dehydroaripiprazole. After validation the method was applied to the 15 authentic hair samples. Positive aripiprazole concentrations >LOQ were found in 93% of the hair samples while 87% of the hair samples were positive for dehydroaripiprazole. The 10th–90th percentiles of aripiprazole concentrations in all hair segments (n=70) ranged from 0.021 to 9.7 ng/mg with median of 1.4 ng/mg and mean of 3.7 ng/mg while the 10th–90th percentiles of dehydroaripiprazole concentrations in all hair segments (n=63) ranged from 0.016 to 11 ng/mg with median of 1.8 ng/mg and mean of 3.5 ng/mg. Highest or similar concentrations were observed in the proximal segments with 10th–90th percentiles of 0.055 to 12 ng/mg, a median of 3.6 ng/mg and mean of 5.3 ng/mg for aripiprazole (n=13) and 10th–90th percentiles of 0.046 to 11 ng/mg, a median of 3.7 ng/mg and mean of 4.9 ng/mg for dehydroaripiprazole (n=12). The 10th–90th percentiles of the drug/metabolite ratio in all hair segments ranged from 0.75 to 3.1 with a median ratio of 1.5. Patient compliance was observed in a number of cases, but a small tendency for wash out effect was also observed.

Discussion
This study reports satisfying validation results for the extraction and analysis of aripiprazole and dehydroaripiprazole in hair. Furthermore, the method enabled successful determination of aripiprazole and dehydroaripiprazole in hair segments from deceased psychiatric patients in which aripiprazole intake was known. The present study has contributed to the establishment of reference levels for aripiprazole and dehydroaripiprazole in authentic hair from chronic users.
Abstract ID 455

Postmortem distribution of paraquat and its metabolites (MP, monoquat) in paraquat poisoning death cases by HPLC-MS/MS.

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Aim

Paraquat poisoning death cases were popular in China. At present, the research on forensic toxicokinetics of paraquat is concentrated on paraquat itself, the related studies on metabolites of paraquat have not been reported. Supported by the National Science and Technology special project work (2015FY11400) and National Key R&D Project (2017YEC0803504, 2018YFC0807403), the study was to investigate postmortem distribution of paraquat and its metabolites in paraquat poisoned death cases.

Methods

Samples of five paraquat poisoning death cases were collected from forensic medicine identification center of Shanxi Medical University from 2016 to 2019. Biological samples were precipitated with acetonitrile or methanol, and the mobile phase was water containing 0.1% formic acid and acetonitrile containing 0.1% formic acid. The linear range and LOD of paraquat and its metabolites (MP, monoquat) in blood, urine and liver were as follows: paraquat: the linear range were 1-1000ng/mL, 1-500ng/mL, 1-1000ng/mg, the LOD were 0.34ng/mL, 0.23ng/mL, 0.53ng/mg; MP: the linear range were 1-1000ng/mL, 1-1000ng/mL, 1-1000ng/mg, the LOD were 1.32ng/mL, 0.60ng/mL, 0.60ng/mg, monoquat: the linear range were 1-400ng/mL, 1-500ng/mL, 5-800 ng/mg, the LOD were 0.58ng/mL, 0.94 ng/mL, 1.25 ng/mg, the concentration of paraquat and its metabolites of tissue and body fluids were analyzed by an MRM HPLC/MS/MS.

Results

Results: Paraquat and its metabolites were detected in the body fluids and tissues of poisoning death cases. The order of which was as following (ng/mL or ng/g): Case1: PQ content: lung(3606.15), blood(3034.09), Stomach contents (1733.20), liver(563.82); monoquat content: blood(20.88),> Stomach contents(5.88), >liver (1.02),>liver (0.53); MP content: blood(38.13), lung(2.53), liver(0.53).Stomach contents (not detected). Case2: PQ content: bile(6121.52), urine(5267.39), kidney(2532.50), pericardial effusion (2058.90), muscle(1914.63), heart blood(1841.56), lung (1541.90), liver (1527.89), heart(1475.56), spleen(1452.96), Venous blood(972.69), Stomach contents (876.16), brain(696.96)> Vitreous humor(164.01); monoquat content: urine(15.02), muscle (11.18), liver(11.73), spleen(10.66), heart(7.58), Stomach contents (6.25), kidney(4.46),>lung(3.56),>heart blood(2.87), Venous blood(2.28),brain(1.19),>bile(0.75), Pericardial effusion(0.45), Vitreous humor(not detected); MP content: urine(41.19), muscle(4.01), spleen(3.44),kidney(3.30),Venous blood (1.84),heart blood(2.17),liver(1.75),Pericardial effusion(1.56),bile(1.46),>lung(1.23),heart, brain, Stomach contents(not detected). Case3: PQ content: muscle(2491.77), heart blood(2233.15), peripheral blood (1596.59), heart(839.62),> cerebrospinal fluid(465.95), bile(214.54),urine(3.53), vitreous humor(not detected); monoquat content: heart blood(3.97), heart(3.39), peripheral blood (2.02), bile(2.00) ->muscle(1.22), urine(0.77), cerebrospinal fluid(0.29), vitreous humor(not detected); MP content: heart(11.60), heart blood(6.89), bile(5.40), muscle(0.98), vitreous humor, urine, bile(not detected). Case4: PQ content: Stomach contents(579.58), heart blood (305.93), kidney(115.07), liver(94.57), urine(83.12); monoquat content: liver (0.71), urine (0.58), heart blood(0.12), kidney(0.11), stomach contents(not detected); MP content: urine (0.21), stomach contents, heart blood, kidney, liver(not detected).

Discussion

Post-mortem redistribution refers to the process of changing the concentration of drug poisons in the corpse, especially the process of changes in the concentration of heart blood-poisoning substances, which is position-dependent and time-dependent. The occurrence of post-mortem redistribution is related to factors such as post-mortem diffusion, post-mortem microbes, post-mortem blood flow, and post-mortem poison degradation. It is generally believed that drug poisons with strong fat solubility and large apparent volume of distribution are more likely to be redistributed after death. The greater the ratio of heart blood/peripheral blood, the more likely this phenomenon occurs.In the results of cases two and three, the concentration ratio of paraquat and metabolites in the heart blood was much higher than that in the peripheral blood (venous blood), and some of the ratios were higher than 1.5. The animal experiment in this study ruled out the post-mortem position of paraquat. The possibility of redistribution indicates a time-dependent post-mortem redistribution of paraquat and its metabolites. Although the apparent volume of paraquat is 1.2~1.6L/kg, since paraquat is a bipyridine compound, it is lipophilic and easily crosses the biological barrier. This may be the reason for its post-mortem redistribution.

Conclusions

Paraquat and its metabolites were detected in paraquat poisoning human tissues for the first time, and the distribution were uneven, which showed a location-dependent post-mortem redistribution.
Abstract ID 460
The study on post-mortem distribution of carbofuran and its metabolites in rabbits.

Ying Wang
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Aim
Carbofuran is a kind of carbamate insecticides, which is unstable in vitro and can be transformed to carbofuran-7-phenyl glucuronic acid (Glu-7PH) in vivo, the phase II metabolite of carbofuran. According to the papers we can know that Carbofuran-phenol can be further metabolized to carbofuran-7-phenyl glucuronic acid (Glu-7PH) in vivo, the phase II metabolite of carbofuran but also the product of carbofuran degradation. It can not be the specific bio-marker for carbofuran poisoning. According to the papers we can know that Carbofuran-phenol can be further metabolized to carbofuran-7-phenyl glucuronic acid (Glu-7PH) in vivo, the phase II metabolite of carbofuran, both in human and animals. There is no systematic study on the substance. In forensic practice, the problem of identifying whether someone is taking poison before birth or given drugs after death is hard, especially the unstable chemicals like carbofuran. Forensic toxicokinetics is an efficient way to solve it. The objective of the research is to identify if carbofuran-7-phenyl glucuronic acid can be a bio-marker for carbofuran poisoning by studying the postmortem distribution of carbofuran and its metabolites. Our team have established efficient methods to detect these chemicals.

Methods
In our experiments, 24 healthy rabbits were randomly divided into 4 groups, one of which was the group of blank control, and the other three groups were administered with 1/2LD50, LD50, 2LD50 carbofuran granules by oral injection. Samples were removed as soon as the pulse and breath were stopped, which include heart, liver, spleen, lung, kidney, brain, muscle, and blood. The content of carbofuran, carbofuran-7-phenol and the glucuronide was analyzed for each sample.

Results
Carbofuran was not detected in the liver and brain of 1/2LD50 group. The content of carbofuran in heart and lung is higher than other organs. Kidney has the highest content of carbofuran-7-phenol for all three dosages. Carbofuran-7-phenyl glucuronide was not detected in the spleen of 1/2 LD50 group, the content of Glu-7PH in the other organs is as follows:

kidney(395.66±167.14ng/g)>muscle(119.20±134.50ng/g)>liver(84.76±63.17ng/g)>brain(48.25±22.41ng/g)>lung(37.98±15.95ng/g)>heart(37.22±12.69ng/g)>heart blood(35.51±14.51ng/ml); The Glu-7PH concentration of the LD50 group in the order of high to low is: kidney (798.11±506.41ng/g)> liver (191.07±147.95ng/g)> muscle(109.02±62.55ng/g) > lung (107.97±113.80ng/g)> heart (102.51±80.01ng/g)> heart blood (70.40±44.34ng/ml)> brain (51.73±41.92ng/g)> spleen(45.68±29.92ng/g); The Glu-7PH content of 2LD50 group from high to the bottom is: kidney(2646.55±1087.03ng/g) > liver(821.80±527.51ng/g) > heart (281.02±125.94ng/g)> lung (250.19±109.38ng/g)> heart blood (205.31±79.88ng/ml)> spleen (191.81±115.76ng/g)> Muscle(100.60±53.33ng/g)> Brain(39.46±18.21ng/g).

Discussion
According to the literature, it can be seen that the metabolic efficiency of carbofuran with rabbit liver microsomes is more similar to human than other animals, so this experiment used rabbits as experimental animals. There have been some studies on the postmortem distribution of carbofuran and its phase I metabolites in vivo, but there is no one about Glu-7PH. The result from this study is consistent with the results of previous research, that is, Carbofuran is high in tissues with abundant blood flow such as heart and lung. The detection of Glu-7PH in different tissues indicates that Glu-7PH can be used as one of the biomarkers for carbofuran poisoning. The absorption, distribution, metabolism, and excretion of carbofuran are very rapid. The original drug may not be detected in some forensic cases of carbofuran poisoning. Glu-7PH is more sensitive than carbofuran and carbofuran-phenol in low dose touch of carbofuran. In the following experiments, the model of post-mortem exposure of rabbits can be designed to estimate the content of Glu-7PH in different tissues and further verify the specificity of Glu-7PH as a marker of carbofuran poisoning.

Conclusions
Above all, Carbofuran is high in blood-rich tissues such as heart and lung, and its metabolites are mainly excreted in the form of glucuronic acid conjugate through the kidney. Carbofuran-7-phenyl glucuronide was detected in all doses, so the conjugate can be used as a biomarker for the identification of carbofuran poisoning.
Study on the stability of EtG and EtS in dried blood spots preserved at different temperatures

Yuping Lu

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Aim

In recent years, the consumption of liquor has been increasing in China, and traffic accidents caused by drinking have become a serious social problem. Supported by the National Science and Technology special project work (2015FY11400) and National Key R&D Project (2018YFC0807403), this experiment establishes a method for determining the ethyl glucuronide (EtG) and ethyl sulfate (EtS) by LC-MS/MS, and studies the stability of EtG and EtS in dried blood spots (DBS) preserved at different temperatures. It provides an experimental basis for forensic identification of ethanol-related cases.

Methods

Eight volunteers drank 0.68g/kg body weight of liquor one hour after a meal. The venous blood was collected after three hours and taken 100ul blood to make DBS, which were divided into three equal groups preserved at 20°C, 4°C, -20°C. The DBS were cut up and extracted with methanol. The concentrations of EtG and EtS in above samples were detected by LC-MS/MS with MRM at 0d, 5d, 9d, 14d, 20d, 23d, 26d, 44d, 50d.

Results

The calibration curves of EtG and EtS were linear in the concentration range of 10ng/ml ~ 1000ng/ml, the correlation coefficient (r) were all more than 0.998, the lower limits of detection were 40ng/ml and 20ng/ml. The initial concentration of EtG was 138ng/ml and the initial concentration of EtS was 194ng/ml. The concentrations of EtG and EtS in DBS were decreased at different temperatures, the decomposition was the fastest at 20°C and the slowest at -20°C. The limited detection time of EtG was 26d ~ 44d and the limited detection time of EtS was more than 50d.

Discussion

The method has high sensitivity, recovery and good repeatability. In this experiment, the DBS by collecting venous blood of drinking volunteers to simulate the residual blood spots on the scene of traffic accidents. The decomposition kinetics of EtG and EtS accorded with single-compartment model. The half life of decomposition of EtG and EtS at 20°C, 4°C, -20°C were 9.57±2.10d, 17.03±2.56d, 18.95±3.40d; 18.67±5.97d, 27.75±5.64d, 36.53±4.33d.

Conclusions

EtG and EtS were decomposed in DBS preserved at different temperatures. The DBS used for forensic identification of the ethanol-related cases should be frozen and be analyzed as soon as possible. Both EtG and EtS are detected simultaneously to reduce the false-negative rate.
Abstract ID 462
Analyzing intact carbofuran-7-phenyl glucuronic acid in biological samples by LC-MS/MS.
Ying Wang
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Aim
Carbofuran is one kind of carbamate insecticides, which is metabolized to carbofuran-phenol in vivo. In vitro, according to the papers we know that carbofuran is unstable and can be transformed to carbofuran-phenol by UV, temperature, and other factors. Carbofuran-phenol is not simply the phase I metabolite of carbofuran but also the product of carbofuran degradation. Thus, it cannot be the specific bio-marker for carbofuran poisoning in forensic practice. It have been validated that carbofuran-phenol can be metabolized to carbofuran-7-phenyl glucuronic acid, the phase II metabolite of carbofuran, both in human and animals. There is no systematic study on the substance. Carbofuran-7-phenyl glucuronic acid is indirectly detected by enzymatic hydrolysis in a few papers. The objective of the research is to establish a detection method of intact carbofuran-7-phenyl glucuronic acid in biological samples by LC-MS/MS.

Methods
1ml Blood or 1g liver samples from human and rabbits were added in 1ml water and then precipitated with 6ml acetonitrile. The mixture sit at 4°C for 20min and centrifuged at 5,000r/min for 20min. ZORBAX HILIC Plus (4.6x100 mm, 3.5 μm), a hydrophilic column, was used to analyze. The mobile phases were water and 0.1% formic acid in acetonitrile, gradient elution. Then the eluant was detected in multiple reaction monitoring (MRM) mode with negative ionization, scanning for 8 min. A case of carbofuran poisoning is analyzed with the method.

Results
The calibration curves of carbofuran-7-phenyl glucuronic acid in the blood and liver is 20ng/ml~5,000ng/ml and 50ng/ml~5,000ng/ml, respectively. The linear relationship is good, regression coefficient R is greater than 0.999, and the limit of detection in blood and liver is 10 ng/ml and 9.93ng/ml, the limit of quantification is 15.63ng/ml and 16.56ng/ml, respectively; the intra-assay and inter-assay precision is 1.53%~5.02% and 5.06%~6.85%; the relative recovery rate is 86.13%, ~92.71% and 87.01%~94.06%. A case of carbofuran poisoning is analyzed, the blood content of carbofuran and carbofuran-phenol is 770n/ml and 29,650ng/ml. The concentration of carbofuran-7-phenyl glucuronic acid in blood is 1,059ng/ml, while in the liver is 4,056 ng/ml.

Discussion
The qualitative and quantitative ions are defined by the law of ion cleavage. The conjugate was detected in a human sample of carbofuran poisoning. Currently, there is no report on the direct analysis of carbofuran-7-phenyl glucuronic acid. This is the first one to detect the phase II metabolite of carbofuran, which provide condition for further work on the forensic toxicokinetics of the chemical.

Conclusions
The method can meet the requirement of analyzing carbofuran-7-phenyl glucuronic acid in biological samples, due to its high specificity and sensitivity.
Abstract ID 463
Study on the stability of EtG and EtS in human blood preserved at different conditions.

Yuping Lu
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Aim
Traffic accidents and related cases caused by drinking are common all over the world. Supported by the National Science and Technology special project work (2015FY11400) and National Key R&D Project (2018YFC0807403), this experiment studies the stability of EtG and EtS in human blood preserved at different conditions. It provides an experimental basis for forensic identification of ethanol-related cases.

Methods
Eight volunteers drank 0.68g/kg body weight of liquor one hour after a meal. The venous blood was collected after three hours and divided into nine equal groups preserved at 20°C, 4°C, -20°C, 20°C (sodium fluoride), 4°C (sodium fluoride), -20°C (sodium fluoride), 20°C (Escherichia coli), 4°C (Escherichia coli), -20°C (Escherichia coli), respectively. The 100ul blood was added 20ul internal standard mixture (EtG-d5, EtS-d5, 50ug/ml, 25ug/ml) and extracted with acetonitrile. The concentrations of EtG and EtS in above samples were detected by LC-MS/MS with MRM at 0d, 2d, 4d, 7d, 15d, 21d, 27d, 33d, 38d, 55d, 62d.

Results
The initial concentration of EtG was 146ng/ml and the initial concentration of EtS was 204ng/ml. The concentrations of EtG and EtS in human blood were decreased at different conditions, with the fastest decomposition at 20 °C and the slowest decomposition at -20 °C. The limited detection time of EtG was 27d ~ 38d and the limited detection time of EtS was more than 62d.

Discussion
EtG and EtS in preserved specimens were found to be decomposed. At the same temperature, EtG decomposed the fastest in the blood added with Escherichia coli, and it decomposed the slowest in the blood added with sodium fluoride. The Escherichia coli had little effect on the decomposition of EtS at different temperatures.

Conclusions
The Escherichia coli can accelerate the decomposition of EtG, but it had little effect on the decomposition of EtS. The sodium fluoride can reduce the decomposition of EtG and EtS. Therefore, EtS is a reliable biomarker of ethanol consumption. In the process of submitting cases, the blood sample should be frozen and preserved after adding sodium fluoride. Both EtG and EtS should be detected simultaneously to avoid the false-negative results caused by putrefaction.

Abstract ID 474
Validation of a Method to Screen for Drugs in Blood and Urine Using Liquid Chromatography Quadrupole Time-of-Flight (LC-QToF) Mass Spectrometry.

Alex Lawson
Alex Lawson, Donna Vincente, Stephen George
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Aim
The rise of novel psychoactive substance use over the last 10 years has meant that toxicology laboratories are asked to detect an ever changing catalogue of substances using highly sensitive techniques. High resolution mass spectrometry is seen as the solution to this problem and has been implemented in a number of laboratories, replacing more traditional screening technologies. Here we present the validation of a LC-QToF MS method to screen for drugs in blood and urine.

Methods
Blank blood or urine was spiked with 240 over-the-counter, prescription and illicit drugs at a number of concentrations. Urine samples (200 µL) were extracted by solid phase extraction and blood samples (50 µL) extracted by protein/phospholipid removal. Extracts were analysed using a Waters G2-XS system running UNIFI in both positive and negative ionisation modes. Drugs were identified by retention time, mass and characteristic fragment ions when compared to a library. Criteria for positive identification of drugs were a retention time (± 0.1 min) when compared to the Waters Toxicology Library and mass error (± 5 ppm) when compared to the assigned molecular formula at low energy. At high energy, presence of at least one accurate fragment ion present in the Waters Toxicology Library is needed for further confirmatory purposes. Intensity of the low energy peak had to be > 1,000 counts. Limit of detection was the lowest concentration that these criteria could be met. To validate the use of the method, the existing screening approach in use at Heartlands Hospital was compared to the LC-QToF MS based methodology by analysis of 70 blood and 42 urine samples from 70 post mortem cases.

Results
The vast majority of drugs (95%) screened were detectable at levels of 10 µg/L in blood and urine with intensities recorded suggesting that many would be detected at much lower levels. The LC-QToF MS approach detected 231 more drugs in the 132 post mortem samples analysed than the existing screening approach and did not miss any drugs previously detected. No carry over was observed when a blood sample containing 10 mg/L of clozapine, cocaine, codeine, diazepam and tramadol was extracted and analysed.

Discussion
The screening protocol outlined here allows for rapid, sensitive and untargeted analysis of a broad range of compounds in blood and urine. Implementation will allow for a simplification of the screening protocol in use, leading to faster turnaround times and improved patient care.

Conclusions
Abstract ID 483

The postmortem distribution of paraquat and its metabolites (MP, monoquat) in paraquat poisoning death dogs.

Hongjuan Ma

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Aim

Paraquat poisoning cases have been occurred frequently in recent years, and the main research focuses on paraquat, but studies on post-mortem distribution of paraquat metabolites have not been reported. In this paper, the distribution of paraquat and its metabolites in infected animals after oral gavage were studied, which was used to guide the sample extraction of paraquat poisoning cases. Supported by the National Science and Technology special project work (2015FY11400) and National Key R&D Project (2017YEC0803504,2018YFC0807403).

Methods

The dogs were received 1/2LD50 dose of paraquat solution (LD50 =34.116 mg/kg) by an intra-gastric administration. Samples of tissues and body fluids of dogs were collected immediately after the dogs poisoning death. The paraquat and its metabolites (MP, monoquat) were all analyzed by HPLC-MS/MS.

Results

The calibration curves of paraquat and its 2 main metabolites (MP, monoquat) in blood were linear in the concentration range of 1~1000ng/mL, the correlation coefficient (r) were all more than 0.9996, the lower limit of detection were0.34~1.32ng/mL, the accuracy were 91.25%~113.44%, the intra-day and inter-day precision were 1.51%~3.99% and 1.92%~4.93%. After paraquat poisoning, the death time is as short as 23 hours and the longest is 8.96 days. The postmortem distribution of paraquat and its metabolites is as follows(ng/mL or ng/mg): PQ content: urine(9609.37) > kidney (2113.68) > lung (2106.59) > spleen (1425.89) > heart (1246.62) > testis (1119.28) > liver (944.65) > stomach (879.29) > brain (671.94) > muscle (658.34) > vitreous humor (387.41) > peripheral blood (311.89) > heart blood (296.47). Monoquat content: urine (202.63) > kidney (140.16) > spleen (67.07) > bile (45.04) > muscle (16.23) > testis (12.66) > lung (12.27) > stomach (11.83) > heart (11.59) > liver (10.49) > vitreous humor (3.60) > heart blood (2.32) > brain (1.45) > peripheral blood (1.20). MP content: kidney (879.11) > lung (366.83) > urine (212.45) > heart blood (185.89) > Peripheral blood (165.89) > heart (150.55) > spleen (148.14) > muscle (116.79) > liver (115.83) > stomach (93.96) > testis (26.88) > bile (23.44) > brain (22.79) > vitreous humor (0.84).

Discussion

Paraquat has a higher concentration in bile, urine and spleen, and is lower in heart, peripheral blood and vitreous humor; it accumulates in the lung after it enters the body, most of it is excreted in the urine in the form of protoplasts within 48 hours. MP has a higher concentration in kidney, lung and spleen, and lower concentration in brain, vitreous humor, bile and stomach; monoquat has a high concentration in urine, kidney and spleen, and is lower in brain, vitreous humor, and heart blood. Monoquat has not been detected in the brain or vitreous humor of some infected animals that died within 48 hours. The postmortem distribution of the two metabolites is similar, both are high in the kidneys and urine; paraquat is mainly excreted by the kidneys; while paraquat is low in the blood,10 to 90 times lower than lungs, so the metabolites in the heart blood, peripheral blood is also relatively low. Paraquat has two metabolic pathways in animals. According to this experimental study, the concentration of MP in tissue organs and body fluids is significantly higher than that of monoquat after oral administration of paraquat solution, therefore, the MP metabolic pathway should be its main metabolic pathway in experimental animals. In the detection of paraquat poisoning cases, if the sensitivity of the instrument is poor, or the poisoning time is slightly longer, it may not be detected in the blood of paraquat and its metabolites. Therefore, it is not correct to take only blood samples in related poisoning cases.

Conclusions

Paraquat and its metabolites (MP, monoquat) can be detected in all tissues and body fluids of paraquat poisoning death dogs, but the distribution were all uneven. The high concentration of paraquat and its metabolites were in bile, urine, and kidney, respectively. In the forensic identification of paraquat poisoning cases, in addition to heart blood and peripheral blood, samples of kidney, lung, liver, urine and bile, should also be collected for toxicological analysis.
Abstract ID 486
Was it drug-induced death? A fatal case with MPHP in the background.
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Aim
4-methyl-α-pyrrolidinohexanophenone (MPHP, PV4) is a new psychoactive substance with pyrovalerone-like properties. We report a case of a 29-year-old male, a drug addict, who died suddenly during police intervention. The autopsy did not provide any evidence to determine a cause and mechanism of death. Because of the high level of agitation, aggression and abnormal behaviour of a deceased prior to his death, a consumption of psychoactive substances was highly suspected.

Methods
During the post-mortem examination whole peripheral blood and urine samples were collected and submitted for toxicological examination. Preliminary toxicological analysis of blood and urine was performed using enzymatic methods. All samples were screened by gas chromatography-mass spectrometry (GC-MS), liquid chromatography-tandem mass spectrometry (LC-MS/MS) and quantified by specific GC-MS method.

Results
Analyses of biological material revealed the presence of MPHP in following concentrations: 0.315 µg/ml in blood and 2.012 µg/ml in urine. Screening for other drugs of abuse and alcohol was negative.

Discussion
Chemical-toxicological analyses of blood and urine revealed the presence of MPHP, a new psychoactive substance, causing pharmacological effects similar to those observed after amphetamine use. Despite the absence of lethal concentrations values of MPHP, in the reviewed case, considering the clinical condition of the man before death as described in the witness statements and medical history, it should be assumed that there is a direct relationship between the use of MPHP and his death. Additional factors contributing to death were excessive physical effort during fight with police officers and paramedics attempting to subdue the man, and the accompanying stressful situation.

Conclusions
The complex case analyses suggest drug-induced death as a result of acute intoxication with MPHP.
Abstract ID 487
The toxicokinetics of paraquat and its two metabolites (MP, monoquat) in paraquat poisoning dogs.
Hongjuan Ma
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Aim
At present, the toxicokinetic studies of paraquat are concentrated on paraquat, related studies on its metabolites (MP, monoquat) in the body have not been reported. The toxicokinetics equations and parameters were obtained by studying the toxicokinetics of paraquat and its metabolites, and the forensic toxicological properties were inferred for the concentration inference at the time of poisoning. This research was supported by the National Science and Technology special project work (2015FY11400) and National Key R&D Project (2017YEC0803504, 2018YFC0807403).

Methods
The dogs were all received 1/2LD50 dose of paraquat dilute solution by an intra-gastric administration, 1 mL of blood was respectively taken from the femoral vein at the time of 0, 10, 15, 30, 45, 60, 75, 90 minutes, 2, 3, 4, 5, 8, 12, 24 hours and 2, 3, 4, 5, 6, 7 days after infected. Paraquat and monoquat were extracted by acetonitrile, MP was extracted by methanol, there were all separated by C18 column, analyzed by HPLC-MS/MS in MRM mode with positive ionization, with a linear range of 1~1000 ng/mL and LOD of 0.34~1.32ng/mL.

Results
Due to individual differences in experimental animals, some always survived and some died after oral administration of paraquat. Survival group: The peak times of paraquat, monoquat and MP in the blood of the infected dogs were 83.28±9.36, 131.39±8.74, 87.43±7.75min, respectively. The peak concentrations were 1837.73±113.69, 3.06±0.19, 264.62±12.4722ng/mL, the elimination half-lives were 36.93±82.27, 875.45±1005.01, 80.98±6409.61min, respectively. Death group: The peak times of paraquat, monoquat and MP in the blood of dogs were 62.40±3.23, 89.55±15.08, 61.25±3.16min, respectively. The peak concentrations were 1519.67±39.4780, 0.98±0.07, 1288.16±32.63ng/mL. The elimination half-lives were 83.69±5915.01, 875.45±1005.01, 80.98±6409.61min, respectively.

Discussion
After paraquat enters the body, a large part of it is excreted in the form of urine by the original form, only a small part is metabolized in the body; the metabolites of paraquat in the animal are MP, DP, MINA and monoquat. Oxidation of one pyridine ring forms MP; demethylation of the pyridine ring forms monoquat; cleavage of the pyridine -pyridine bond produces MINA. Toxicokinetics is a cross-edge discipline that applies the principle of pharmacokinetics to explore the development of the toxicity or adverse effects of drugs and other exogenous chemicals. At present, the toxicokinetic studies on paraquat are concentrated on paraquat, and the toxicokinetics of its metabolites have not been reported yet. In this experiment, dogs were used as the research object. Oral gavage was used to simulate the routine poisoning method. The blood at different time points was collected to study the toxicokinetics of paraquat and its two main metabolites (MP, monoquat). The main forensic toxicokinetic parameters (K01, K10, t1/2 K10, V1, V2, CL, T max, C max) of the two groups were statistically analyzed by SPSS 20.0 statistical software. Statistical results showed that there were statistical differences in paraquat: K01, K10, t1/2 K10, CL; MP: K10, t1/2 K10, V1, CL, Tmax statistically significant; monoquat: There was a statistical difference between t1/2 K10 and C max. The difference in survival time may be due to the circadian rhythm of the organism, the difference in exposure time, and the difference in sensitivity of the infected animal to the same drug poison.

Conclusions
Paraquat, monoquat, and MP all conform to the first-stage absorption two-compartment open model in the blood, which can provide experimental basis for the estimation of the concentration of toxicants in the body of paraquat poisoning.
Abstract ID 497
Contact points of forensic medicine and psychiatry illustrated with fatalities related to new psychoactive substances (NPS)
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Aim
The aim of this study is to find an interaction between the use of new psychoactive substances (NPS) and the cause of death, taking into account information indicating the psychological disorder of the victim before death.

Methods
The research material includes the results of chemical-toxicological analyzes of the post-mortem blood collected at the autopsy of 40 people, whose cause of death was related to the use of new psychoactive substances (NPS).

Blood samples were subjected to extraction in the solid-liquid system (SPE), and then analyzed by high-performance liquid chromatography with mass detection for identification, followed by quantitative analysis, gas chromatography with mass detection (GC-ESI-MS) and high-performance liquid chromatography coupled tandem with a mass detector (LC-ESI-MS/MS). Information on individual cases comes from the files of cases made available by the prosecutor's office for scientific purposes.

Results
In the course of the study, 24 types of NPS were detected, including synthetic cathinones - 15 cases, synthetic cannabinoids - 10 cases, synthetic opioids - 13 cases, others - 2. The concentration of xenobiotics determined in blood represented large ranges of concentration.

In the study group there were 5 women (12.5%) and 35 (87.5%) man. The victims were at 16-58 years old. In the group of victims - 10 (25%) suicides were indicated (hanging, jumping from high altitude, self-injury and exsanguination, acute drug intoxication), the rest cases – 30 (75%) were accidental deaths. In 6 cases medicines used to treat mental disorders were found which suggested that the victims were struggling with mental problems before death. As it was implied by the available information in the presented material, more than 50% of the victims had mental problems before death.

Discussion
Drug use and mental illness may have a common substrate or may be a coincidence. An NPS action on human health, especially in the long time interval, is still hardly known. These restrictions impose, among other things, the enormous challenge of diagnosis and therapy to psychiatrists and psychotherapists. It is important to emphasise that the invention of the NPS creators always precedes knowledge of the characteristics of the relationship and the consequences of its use.

Conclusions
If NPS is taken, the principle of risk assessment is applied and the opinion on the unpredictability of effects should be adopted as a standard.
Abstract ID 505  
Development of an analytical method to detect pesticides in biological samples of Giant Anteater by liquid chromatography-tandem mass spectrometry (LC-MS/MS).  
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Aim  
Pesticides are products intended for use in the production, storage and processing of agricultural products, in order to preserve them from damaging action of living beings considered harmful. Pesticides can contaminate water and soil, as well as accumulate in the biota, through the food chain, and may cause ecological imbalance in the environment where they are applied. The Brazilian Cerrado is a biome formed mainly by forest formations, savannas and pastures, and it has been under changes in the last years due to the human occupation, which can be described by the expansion of new areas, including deforestation, construction of new highways and cultivations. The mostly common agricultural crops in Brazilian Cerrado are grains, eucalyptus, sugar cane and pastures. Giant anteater (Myrmecophaga tridactyla) is an animal that lives in the Brazilian Cerrado. It is considered vulnerable according to an International Union for Conservation of Nature and Natural Resources (IUCN).  

Methods  
For sample preparation, 250 μL of horse defibrillated whole blood was transferred to polypropylene tube, followed by 25 μL of internal standard (diazepam-d5, 200 ng/mL in methanol) and 500 μL of iced-cold acetonitrile. After 5 min of resting, 100 mg of QuEChERS salt (Q-sepTM, Restek®) was added. The tube was capped, agitated for 10 min and centrifuged at 18,406 g/10 min. The organic phase (125 μL) was transferred to a 96-well plate already filled with 375 μL of aqueous mobile phase. Two microliters were injected onto a Raptor™ Biphenyl column (100 mm x 2.1 mm, 2.7 μm, Restek), with mobile phase composed by (A) water and (B) methanol, both containing 0.1% of formic acid and 2 mmol/L of ammonium formate (gradient elution). The flow rate was set to 400 μL/min, with 15 min run time. The mass spectrometer was equipped with an electrospray ionization source (ESI), operated in positive and negative mode. The source parameters optimized were: heat block temperature 400 °C; ion spray voltage 4.0 kV for positive substances and -3.0 kV for negative substances; nebulizer gas (N2) flow 3 L/min; desolvation line temperature 250 °C; drying gas (N2) flow 10 L/min; heating gas (N2) flow 10 L/min; and collision induced dissociation gas pressure (Ar) 270 kPa. The analyses were performed in multiple reaction monitoring (MRM) mode. For each compound, two MRM transitions were selected, one for quantification and one qualifier for confirmation identification using the MRM ratio as identification criteria (20% maximum tolerance window). Method validation was performed following recommendations from Scientific Working Group for Forensic Toxicology (SWGTOX) for quantitative analysis. Giant anteater blood samples are being collected in the State of Mato Grosso do Sul and includes post mortem samples of roadkilled animals and also samples collected from animals monitored by GPS vests living in the Brazilian Cerrado region.

Results  
A total of 7 pesticides (2,4-D, aldicarb, atrazine, carbofuran, chlorpyrifos, fenthion and mevinphos) and a transformation product of aldicarb (aldicarb sulfone) were optimized to be analyzed in the MRM mode. The limits of detection and quantification of these analytes were determined to be 5 ng/mL (25 ng/mL for 2,4-D). The linearity was evaluated from 5 to 1000 ng/mL (except for 2,4-D: 25 to 5000 ng/mL), and shown good performance (r > 0.99, 1/x2). Method precision (calculated by ANOVA) and accuracy were greater than 20%. Until now, 64 real samples (27 from roadkilled animals and 37 from animals monitored by GPS vests) were analyzed, and none of the selected compounds were found.

Discussion  
The proposed method presents advantages over other conventional extraction methods, because uses a small amount of solvent and is a fast execution method. This study is of great relevance because it will help understanding where, how and when the animals are being impacted by the anthropogenic advance through the toxicological analyzes.

Conclusions  
The following validation parameters were evaluated for 8 selected substances: accuracy, precision, calibration model, limit of detection and limit of quantification. The developed method proved to be effective for detection of substances of different chemical classes and also proved to be a sensitivity method. The next step is to validate the method for other analytes (glyphosate, paraquat dichloride, fipronil, malathion, endosulfan, deltamethrin, permethrin and sulfurlamid) in order to search them in the anteater samples.
Abstract ID 519
Fatal butylone intoxication: a case report.
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Aim
Butylone (2-methylamino-1-(3,4-methylenedioxyphenyl)butan-1-one, β-keto-MBDB) is an entactogen, psychedelic, and stimulant psychoactive drug of phenethylamine class, synthetic cathinone, structurally related to MBDB, MDMA and methylene. Synthetic cathinones are derived from cathinone, the active ingredient of the khat plant Catha edulis. Cathinone derivatives are the β-keto-analogues of a corresponding phenethylamine, often marketed online and in head shops such as “plant food”, “bath salts”, or “research chemicals”, often with a printed warning that they are “not for human consumption”. Synthetic cathinones are commonly abused novel psychoactive substances (NPS) as one of the fastest growing groups monitored by the EMCDDA. The authors present the first case in the Czech Republic where butylone was detected in post-mortem samples, as a result of illicit use.

Methods
A 21-year-old man was found dead in front of the house after falling down from the 8th floor. Amphetamines/Ecstasy were detected in urine by immunoassay CEDIA (Thermo Scientific INDIKO). General unknown screening was performed on serum, urine, tissue and gastric content using a solid phase extraction with GC/MS (Varian 450 GC/220 MS). Butylone was identified in all post mortem samples. LC/MS/MS (Sciex 5500 QTRAP) was used for quantification of butylone in serum and urine, after solid phase extraction. For LC/MS/MS quantification method evaluation, 0.1 mL drug-free serum and urine samples fortified with 0.01-3.0 mg/L of butylone and the addition of deuterated internal standard methylone d3 were processed with solid phase extraction. The extracts were evaporated and reconstituted, then injected into LC/MS/MS system. Chromatographic separation was performed using Phenomenex Kinetex C18 column (50 x 2.1 mm, 2.6 µm particle). The mobile phase was consisted of 10 mM ammonium formate, 2% formic acid and acetonitrile. Mass spectrometric analysis was performed under electrospray ionization in positive ion multiple reaction monitoring (MRM) mode. LOQ=0.01mg/L, LOD=0.001mg/L.

Results
Butylone concentrations in serum and urine were respectively 1.5 mg/L and 283 mg/L. The urine sample was diluted. Ethanol in serum was not found. For interpretation of toxicology results were used data published by the North Carolina Office of the Chief Medical Examiner: butylone: Therapeutic: N/A, Toxic 0.1 mg/L, Lethal: 1.2-20 mg/L: the data have been compiled from previously published scientific literature and from prior the Office experience. The proper interpretation of post mortem drug concentrations is complex and complicated by factors including individual variations in response to drugs, tolerance, physical stature, disease states, the presence of other drugs, and the potential for post mortem changes in serum drug concentrations.

Discussion
Ring substituted cathinones, such as butylone, act as transporter substrates that increase the release of dopamine, serotonin and norepinephrine. Users generally snort, inject, smoke or orally consume synthetic cathinones. The desired effects are euphoria, stimulation, alertness, talkativeness, sexual arousal, focused mind and an overall positive feeling. The effects occur within 30 to 45 min of administration and the desired effects last from 1 to 4 hours. The undesirable side effects are anxiety, insomnia, fatigue, agitation, aggression, panic, disorientation, confusion, hallucinations, memory loss, excited delirium, paranoia, chest pain, tachycardia, breathing difficulties, hyperthermia, hypertension. Many users mention a strong urge to re dose, sometimes tripling the originally planned dose. Crush period persisted for 10-12 hours with headache, loss of sleep and appetite.

Conclusions
The authors present the first case in the Czech Republic where butylone was detected in post mortem samples, as a result of illicit use.
Abstract ID 530
Inert Gas Asphyxiation: A Suicide Case Using Industrial Argon in a Household Setting.

Bronwen Davies
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Aim
Background: Cases of fatal asphyxia due to inert gas inhalation have been widely reported. Many suicidal cases involve securing a plastic bag over the head, with an inflow of gas into the bag, resulting in suffocation due to reduced oxygen availability. The most commonly reported gases used in these cases are helium and nitrogen, with few cases of argon asphyxiation published. This case illustrates the use of industrial compressed argon gas in a formal household setting. The challenges faced in supporting these cases with toxicological evidence, especially in resource-constrained environments, is further discussed.

Methods
The case presented is one of a 55-year-old female, found deceased and supine on a chair in her house. A plastic bag was placed over her head and secured around her neck with tape, with a pipe that fed into the bag. Next to the deceased was a dog, who was also deceased with a plastic bag over its head and a tube extending from the bag. A suicide note was identified on scene, as was a large industrial compressed argon gas cylinder to which the tubing was attached, which may have been available from her work as an airplane engineer.

Results
The autopsy was performed approximately 48 hours after death declaration. Key post-mortem findings included the presence of facial and conjunctival congestion with petechial haemorrhages, pulmonary oedema, pleural petechial haemorrhages, coronary atherosclerosis and subendocardial fibrosis and evidence of a fatty liver and chronic pancreatitis. Sex reassignment surgery (male to female) was also noted. Both lungs were removed in their entirety and submitted with blood for toxicological evaluation. Toxicological analysis by the National Government laboratory was conducted 7 months after collection. No drugs were detected and the analysis for argon in the lungs was negative.

Discussion
While positive detection of inert gases in post-mortem specimens is being increasingly reported; in most cases the cause of death (CoD) is still determined by the physical evidence at the death scene and supporting signs of anoxia at autopsy. In this case, the CoD was determined by the pathologist to be consistent with the history of gassing and the consequences thereof. Given that air-tight tissue sampling was not possible at autopsy, together with a delay in analysis, a loss of the gas cannot be excluded.

Conclusions
The death scene and autopsy findings (despite being non-specific) supported the finding of inert gas asphyxiation, even in the absence of toxicological findings. Supporting the circumstantial findings with toxicological results in service remains a challenge, particularly in resource constrained environments.
Abstract ID 533
The Use of Mortuary Data in Child Death Prevention: The Case of Street Pesticides.
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Aim
South Africa (SA) has still not met child mortality reduction targets for the Millennium Development Goals, despite substantial investments in achieving these. Child mortality has declined and plateaued, but children continue to die from preventable causes of death. Child death review projects targeting the prevention of unnatural child death in SA have become essential. Morbidity and mortality rates related to pesticide exposure are high in lower socio-economic communities, where toxic pesticides are widely available for use. In Cape Town, SA, informal vendors sell highly toxic street pesticides for domestic use. These are mixtures of unregistered pesticides, sold as liquids or granules in unlabelled bottles and packages. While cheap and widely available in informal settlements, these formulations are not regulated and illegal to distribute. The aim of this study was to investigate pesticide-related death in children within the West-Metropole of Cape Town.

Methods
Cases admitted to Salt River Mortuary in Cape Town (West-Metropole of Cape Town drainage area) from 2010 to 2017 (inclusive) were reviewed. A retrospective investigation of suspected and confirmed pesticide-related deaths in child cases (<18 years old) was conducted. Demographic, autopsy, investigative and toxicological data were collected from post-mortem and other investigative reports and analysed.

Results
Thirty-six pesticide-related child deaths were identified, of which 13 were male (36%) and 23 were female (64%). The average age was 8 years old (range: 3 weeks – 17 years). Most deaths were deceased upon arrival (n=12; 33%) or died after admission to a medical center (n=20; 56%). Common symptoms observed in the poisoned patients were pin point pupils, fasciculations, salivation, vomiting and secretions. Suicides were common amongst older teenagers, while accidental deaths occurred largely in under 5-year olds. Two cases of homicide were suspected. Routine toxicology was performed in most cases (94%). The organophosphate terbufos was found to be the most common pesticide detected analytically (64%). Diazinon, methamidophos, phorate and organophosphate metabolites were detected in single separate cases.

Discussion
These cases identified originated from lower socio-economic areas in Cape Town, where the use and availability of pesticides, especially in the form of toxic street pesticides, is wide. There was a clear distinction in the age of decedents in suicide versus accidental cases, with older adolescents having access to pesticides to commit suicide, and younger children or infants accidentally being exposed. Previously, public health groups worked toward the banning of aldicarb (Temik) due to its detection in poisoning cases. This study was the first to identify terbufos as a prominent pesticide involved in child pesticide deaths. This data may be used to support the notification of these cases, aid in improving the training and impact of environmental health practitioners within the communities, and support the banning of terbufos from agricultural use in South Africa.

Conclusions
Collaborative work between forensic medicine departments, prosecuting authorities, police services, pediatric specialists, and social workers in child death prevention is essential. This study illustrates the potential use of mortuary data within community and public health sectors, to identify the role of pesticide toxicity within child death. This may assist in targeted and evidence-based intervention strategies and policy reform to reduce accidental and suicidal mortality associated with acute pesticide exposure in children.
Abstract ID 541
Characterization of NPS metabolites through the analysis of hair samples by high-resolution mass spectrometry. A real case of methoxetamine.
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Aim
Methoxetamine is a dissociative drug classified as an arylcyclohexylamine-type compound, considered as a new psychoactive substance (NPS). It is produced as a ketamine analog in the illicit market. However, methoxetamine presents some chemical differences with respect to ketamine, in which the chlorine group on the phenyl ring is replaced by a methoxy group, and the N-methyl group by a N-ethyl group. There is not much information about metabolism of this compound due to its recent existence. It is considered a fashionable drug that mimics the psychoactive effects of ketamine. The different metabolites proposed are often found in vitro studies or even in real samples where high concentrations in urine samples are expected.

Previously, this case was analyzed as part of a work for the evaluation of NPS as well as other compounds present in hair, using a screening method based on liquid chromatography coupled to high resolution mass spectrometry (LC-MS/MS Orbitrap) in ten real cases. In this real hair sample that we present, it was re-injected the same sample to the study of metabolites of ketamine and other arylcyclohexylamines, as methoxetamine.

The same instrument was used to the study and fragmentation of the possible metabolites present in a hair sample, after searching possible metabolites (phase I) originated from the hydroxylation, dehydrogenation, reduction, N-dealkylation, O-demethylation, and comparing them with the ones that are already described in preexisting literature. This work aimed to study a real case where a high amount of arylcyclohexylamines analogues (methoxetamine and ketamine) were detected to investigate their metabolites by high resolution mass spectrometry (LC-MS/MS Orbitrap).

Methods
The initial determination of ketamine, norketamine and methoxetamine was performed by a screening and fragmentation list of substances with a method using LC-HR-MS/MS Orbitrap. The preanalytical process involved the following steps: (i) hair washing with dichloromethane, (ii) methanolic incubation for 18 hours, (iii) sonication, (iv) centrifugation, (v) evaporation and reconstitution in 100 μL of methanol. Chromatographic separation was performed by a gradient elution with a binary mobile phase consisted of 2 mM aqueous ammonium formiate plus 0.1% formic acid (pH=3, eluent A) and 2 mM aqueous ammonium formiate with acetonitrile:methanol (50:50, v/v; 1% water) plus 0.1% formic acid (eluent B). The flow rate was set to 0.5 mL/min in an Accucore PhenylHexyl column (100 mm x 2.1 mm x 2.6 μm). The instrument was set to positive/negative switching ionization mode using full scan data (FS) and subsequent Data Dependent Acquisition Mode (DDA).

Ketamine and its metabolite norketamine, as well as methoxetamine, were quantified by the high resolution technique of LC-MS/MS Orbitrap, using a linear calibration model (R² > 0.99), weighted 1/x, in the calibration range from 0.025 to 50 ng/mg (n=11).

Subsequently, it was re-injected, with the similar method but with another fragmentation list, for the search for methoxetamine metabolites.

Results
By means the screening method, identification and the quantification of ketamine (36.1 ng/mg), norketamine (4.1 ng/mg) and methoxetamine (70.5 ng/mg) was possible. The similar method was used to analyze the same sample that was re-injected by a screening and fragmentation list of metabolites with a method using LC-MS/MS Orbitrap. Ten different metabolites were identified by o-demethylation, N-dealkylation, reduction and dehydrogenation.

Discussion
Using the initial screening method, it was set to positive/negative switching ionization mode (FS) and (DDA). Where the presence of a series of substances can be identified and quantified by means of FS data and the identification is made by fragmentation obtaining their MS/MS fragmentation spectra. This fragmentation is selective and is carried out by a closed list, so that if the selected ion or ions are found precursors with a high accuracy of mass at certain abundance, they will be fragmented. Through the acquisition mode, FS data of different mass ions can be evaluated if the ions acquired in this way were appropriate in the case that they belong to our own hair matrix, or substances of interest, such as, comparison of exact mass theorical lists of NPS, as well as the search for a list of theoretical and/or known metabolites of the detected mother drug, but not monitored in the initial analysis method.

By means of the reinjection of the same extract, the way of acquiring the method can be varied, and the fragmentation of the compounds to be studied can be carried out, either by their fragmentation from a given abundance mode (DDA) or by a fragmentation independent of their abundance Product Reaction Monitoring (PRM).

Conclusions
Hair samples is suggested as a valid matrix to determine arylcyclohexylamines as ketamine and methoxetamine and its metabolites, as well as for its application to other type of NPS. Considering the matrix and drugs characteristics, ten different metabolites were identified by high resolution techniques.
Abstract ID 546
The first fatality poly-consumption case involving cyclopropyl-fentanyl reported in Madrid.
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Aim
The presence of fentanyl, or structural variants of it, can cause intense sedation, loss of consciousness or fatal human intoxication and death. Cyclopropyl-fentanyl is a structural analogue of fentanyl, classified as a New Psychoactive Substance (NPS). When the reported case was studied in 2017, cyclopropyl-fentanyl was not a controlled substance, but it was under study in risk assessment and control as NPS by European Monitoring Centre of Drugs and Drug Addiction (EMCDDA) due to its toxicity, lethal in many cases, already detected in some countries.

In this work, we report the first fatality case occurred in Spain (December 2017) involving cyclopropyl-fentanyl, including a tissue distribution study of other drugs along with this synthetic opioid. Additionally, we have conducted a structural chemical study of different cyclopropyl-fentanyl metabolites using high resolution Orbitrap mass spectrometry (Orbitrap, LC-HR-MS/MS), including a structural differentiation study of crotonyl-fentanyl, its isobaric analogue, with the assistance of ultraviolet spectroscopy.

Methods
A 24-years-old man was found death at home. The medical examiner initially suspected of poly-drug intoxication as cause of death. Drug paraphernalia was located in his home. The paraphernalia and all biological samples (blood, vitreous humor and urine from the autopsy) were submitted to our laboratory (INTCF) for a comprehensive toxicological screening. Urine was screened by enzyme-immunoassay (CEDIA). The volatile analysis was performed in blood and urine using HS-GC-FID. For screening, confirmative and quantitative analysis GC-MS, HPLC-DAD, LC-MSMS and (Orbitrap, LC-HR-MS/MS were used, using liquid-liquid extraction (LLE) and solid-phase extraction (SPE). The validation was performed for cyclopropyl-fentanyl with solid phase extraction and its quantization by LC-MS/MS method. The linearity in the range of 1-200 ng/mL (n=6), with a linear calibration model (R2>0.99), weighted 1/x. Limits of detection and quantification achieved were 0.5 and 1.0 ng/mL, respectively. The precision of the method was evaluated with spiked samples of negative blood at three levels of concentration, obtaining relative good standards deviations comprised among 5.8% and 10.7%. Accuracy of the method was also good, between -6.7% to 6.6%. The structural differentiation of both compounds (cyclopropyl-fentanyl and crotonyl-fentanyl, which are isobaric compounds), was accomplished with the assistance of ultraviolet spectroscopy (HPLC-DAD).

Results
Heroin (heroin metabolites), cocaine, amphetamines, and related substances, benzodiazepines and cyclopropyl-fentanyl were detected in all biological samples. The fentanyl derivative was detected in blood (20.4 ng/mL), vitreous humor (26.4 ng/mL), and urine (86.1 ng/mL) and metabolites of cyclopropyl-fentanyl (e.g. cyclopropyl-norfentanyl).

From the different non-biological samples (paraphernalia), a lethal mixture consisting of heroin (23%) and cyclopropyl-fentanyl (44%) was detected in one of those analyzed samples.

Discussion
In this case, non-biological samples close to the deceased were sent at express request, and it has been decisive to relate this fatal intoxication with the consumption of this substance, where heroin was also detected as a result of that association.

The identification was made by comparing mass spectrums by electronic impact of the libraries (SWGDRUG and Cayman Chemical).

In all the biological samples and two non biological samples fentanyl derivative was identified, but the differentiation of cyclopropyl-fentanyl from crotonyl-fentanyl was not possible. The cyclopropyl-fentanyl and the crotonyl-fentanyl (with an additional double bound, but same empirical formula) was differentiated by comparison of their ultraviolet (UV) spectra. On the contrary, was not possible to differentiate them by their respective chromatographic retention times because there were no previous standard references, or their mass spectrum using GC-MS and LC-HR-MS/MS.

Conclusions
The use of high-resolution instrumentation (Orbitrap, LC-HR-MS/MS) allowed the identification of the fentanyl derivatives and metabolites with high sensitivity. Although these compounds were not included in the common screening libraries, other different strategies for the detection and differentiation achieved greater efficiency and effectiveness in drug identification.

In this reported case, the availability of urine with high concentration of parent drugs and metabolites, and the paraphernalia, was crucial to clarify this fatality involving common drug abuse, including this NPS fentanyl analogue. According to the medical examiner, it is a violent death of presumably accidental etiology. The cause of death was due to poly-drug abuse, and match with a main role of the fentanyl analogue. The necropsy findings and background allow us to establish a date of death approximately in the afternoon/evening of the previous day.
Abstract ID 273
Potential effects of the co-administration of AMB-FUBINACA and pFPP in New Zealand.

Diana Kappatos
Samantha Coward, Diana Kappatos
ESR

Aim
Since May 2017 more than 80 deaths across New Zealand (NZ) have been linked to the use of the synthetic cannabinoids AMB-FUBINACA and 5F-ADB. These new psychoactive substances have also been the most prevalent synthetic cannabinoids intercepted at the border by NZ Customs, and were detected in plant material submitted by NZ Police. A distinct geographical distribution of these drugs was observed across the country.

The geographical differences were also observed in intoxication cases, with AMB-FUBINACA associated with cases in the northern region of New Zealand’s North Island, and 5F-ADB more observed in the lower half of this island. The effects of the use of these synthetic cannabinoids was found to be rapid and profound, with severe adverse reactions such as seizures and unconsciousness, and death, occurring very quickly.

Interestingly, during analysis of the plant material submitted by NZ Police, ESR found that a proportion of the AMB-FUBINACA positive plant materials, also contained the stimulant para-fluorophenylpiperazine (pFPP). This was not present in plant material containing 5F-ADB. We do not fully understand why pFPP is present alongside AMB-FUBINACA, but it is possible that it was deliberately mixed into the plant material to help manage the severe effects associated with using AMB-FUBINACA on its own. Users of pFPP have reported a delay in the onset of the psychoactive effects after its use.

This study was undertaken to determine the potential effects of co-administration of these two psychoactive substances.

Methods
Biological specimens collected from users of AMB-FUBINACA were analysed by liquid chromatography with tandem mass spectrometric detection (LC-MSMS) and by liquid chromatography with time-of-flight (LC-TOFMS) for the presence of pFPP and a range of synthetic cannabinoids and some of their metabolites. The concentrations of pFPP and the synthetic cannabinoids were determined in the blood specimens.

Results
We have undertaken toxicological examination of blood and urine samples associated with users of AMB-FUBINACA and found pFPP in a number of cases. The levels of AMB-FUBINACA and AMB-FUBINACA metabolite in the blood of persons whose death appeared to be linked with AMB-FUBINACA were compared to those users who were either treated in emergency departments or had been found to be impaired drivers.

AMB-FUBINACA was found to be significantly metabolised in biological samples with a relatively small amount of the parent drug present in the blood and urine, whereas, pFPP itself was detected in the blood and urine. A significant number of cases whose death appears to be linked with AMB-FUBINACA had also used pFPP. The results of this study will be presented.

Discussion

Conclusions
Abstract ID 34
Application of an enantioselective analysis of amphetamine in whole blood in cases of driving under the influence.

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NFI

Aim
In the Netherlands, legal limits on the use of drugs while driving have been enforced since the 1st of July 2017. These limits apply for nine drugs of abuse, including amphetamine. The Dutch law does not discriminate between the illicit drug use of amphetamine (mixture of levo- and dexamphetamine) and the therapeutic use of dexamphetamine. However, some suspects of driving under the influence defend their positive amphetamine blood result by claiming using prescribed dexamphetamine. The aim of this study was to apply an enantioselective analysis of amphetamine in whole blood in these cases of driving under the influence in order to be able to discriminate between claimed prescribed dexamphetamine use and the illicit drug use of amphetamine.

Methods
Whole blood extracts were analysed for the quantification of amphetamine and other drugs of abuse, using a routine UPLC-MS/MS method. When the presence of amphetamine was confirmed and prescribed use of dexamphetamine was claimed, the enantiomeric composition of amphetamine was determined. Separation of the enantiomers was achieved by applying a UPLC-MS/MS method, utilizing a polysaccharide based analytical column (Phenomenex® Lux® 3 µm AMP) in combination with a methanol/ammonium bicarbonate (pH 11) gradient. Detection was performed with a triple quadrupole mass spectrometer (Waters Quattro Premier MS). The lowest concentration that could be measured with this method was 5 µg/l.

Results
In six cases in which routine analyses showed the presence of amphetamine in whole blood, additional enantioselective analysis was performed. In five of these cases, the concentration of levo-amphetamine was 1.5 to 2 times higher than the concentration of dexamphetamine. Only in one case, 95% of the measured amphetamine was dexamphetamine.

Discussion
The enantioselective analysis was applied in six cases in which the driver claimed dexamphetamine use. In one case, 95 % of the measured amphetamine was dexamphetamine which showed that the driver indeed used dexamphetamine. Furthermore the prescribed dose in this case matched the measured concentration. In the other five cases, the results of the enantioselective analysis could not be explained by the use of prescribed dexamphetamine alone. It showed that at least illicit amphetamine was used whether or not in combination with prescribed dexamphetamine. In two of these five cases, the prescribed dose fitted with the measured concentration and without the enantioselective analyses, a distinction between prescribed dexamphetamine and amphetamine as drug of abuse could not have been made. In four other cases where the driver claimed a prescribed use of dexamphetamine, blood samples were already destroyed and no enantiomeric analysis could be performed. In these cases, the amphetamine concentration was estimated based on the claimed dose and compared with the concentration of amphetamine measured with the routine analysis. In three of these cases, the claimed dose fitted with the measured concentration in blood. In these cases, application of the enantioselective analysis could have been of importance.

Conclusions
The enantioselective method for amphetamine in whole blood was successfully applied in cases of driving under the influence. Using this method we were able to discriminate between claimed prescribed dexamphetamine use and the illicit drug use of amphetamine.
Abstract ID 80
Determination of 5-MeO-DIPT in Human Urine Using Gas Chromatography Coupled to high resolution mass spectrometry.
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Aim
5-Methoxy-N,N-Diisopropyltryptamine (5-MeO-DIPT) is a designer hallucinogen derived from tryptamine, and its abuse has been recently rising in popularity. In this study, a qualitative and quantitative method was developed for determination of 5-MeO-DIPT in urine by gas chromatography high resolution mass spectrometry, 5-OH-DIPT and 5-MeO-IPT were identified as 5-MeO-DIPT metabolites in abusers’ urine.

Methods
5-MeO-DIPT was extracted from urine by Liquid-liquid extraction with ethyl acetate under alkaline conditions. The extract was analyzed by GC-Orbitrap-MS in full scan mode, with resolution of 60,000 (FWHM). The established GC-HRMS method was applied to detect 5-MeO-DIPT in suspected abuser’s urine sample, and the metabolites of 5-MeO-DIPT were investigated by GC-HRMS.

Results
The calibration curves was linear (r > 0.994) in the concentration range of 2-300 ng/mL, and the limit of detection was 1 ng/mL. The accuracy and precision were 92.9% - 108.7% and 3.0% - 11.4%, respectively. This method is simple, sensitive, and can be used to qualify and quantify 5-MeO-DIPT in human urine. It has been successfully used to detect 5-MeO-DIPT in drug abusers’ urine.

Discussion
The concentration of 5-MeO-DIPT in abusers’ urine samples were between 1 ng/mL and 2.8 ng/mL. 5-OH-DIPT and 5-MeO-IPT, two urinary major metabolites of 5-MeO-DIPT, were identified in urine samples from 5-MeO-DIPT users. Furthermore, the stability of 5-MeO-DIPT in human urine was investigated. It is discovered that the concentration of 5-MeO-DIPT in urine samples significantly decreased (P<0.05) by 22.8%, 33.2%, 38.2% after they were stored 24h at 25°C, 5 day at 4°C and 7 day at 4°C, respectively. Therefore, it is recommended that urine should be stored under freezing conditions before performing 5-MeO-DIPT analysis.

Conclusions
A method for determination of 5-MeO-DIPT in human urine using gas chromatography coupled to high resolution orbitrap mass spectrometry was developed and validated. This method is simple, sensitive, and suitable for testing 5-MeO-DIPT in human urine. Two major metabolites of 5-MeO-DIPT, 5-MeO-IPT and 5-OH-DIPT were identified in 5-MeO-DIPT abusers’ urine samples as well.

Abstract ID 104
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Aim
4F-MDMB-BINACA (methyl 2-(1-(4-fluorobutyl)-1H-indazole-3-carboxamido)-3,3-dimethylbutanoate) is a newly emerging synthetic cannabinoid. This compound is structurally similar to 5F-ADB (5F-MDMB-PINACA), differing by the removal of one carbon (-CH2) linkage from the carbon tail of the molecule. 4F-MDMB-BINACA was identified in ten blood and urine samples obtained from living persons with suspicion of drug abuse referred to the Istanbul Toxicology Department of the Council of Forensic Medicine (Turkey) by judicial authorities in March 2019.

Methods
The blood and urine samples of the cases were initially subjected to systematic toxicological analysis for alcohol, abused, and therapeutic drugs. Methodologies utilized in the analyses include a headspace-gas chromatography with flame ionization detection (GC–FID) screen for volatile compounds, a liquid chromatography–tandem mass spectrometry screen (LC–MS/MS), a liquid chromatography–time-of-flight mass spectrometry screen (LC–ToF), and a gas chromatography mass spectrometry (GC/MS) for classically abused drugs and therapeutic agents. For the screening of new psychoactive substances, a qualitative LC-HRMS method, which can detect over 130 new psychoactive substances with their metabolites, was used. The samples were prepared using solid phase extraction (SPE) and analyzed by LC-HRMS for synthetic cannabinoid screening. The LC-MS system consisted of an HR Q-Exactive Plus mass spectrometer interfaced to a Dionex Ultimate 3000 RS UHPLC system.

Results
4F-MDMB-BINACA and its metabolites 4F-MDMB-BINACA N-(4-hydroxybutyl) and 4F-MDMB-BINACA butanoic acid were identified by liquid chromatography-high resolution mass spectrometry (LC-HRMS) in blood and urine samples.

Discussion
Two metabolites of 4F-MDMB BINACA have been supplied by Cayman Chemical. But there are no available data about pharmacology, metabolism and toxicity of 4F-MDMB-BINACA in scientific context. This study represents detecting and identification of 4F-MDMB-BINACA and its two metabolites in human blood and urine.

Conclusions
Our report is the first in vivo detection of 4F-MDMB-BINACA in a Turkey. The study indicates a significant worrying alarm about the emergence of this substance.
Abstract ID 107
High Data Quality, 24/7 Productivity and Result Defensibility for Blood Alcohol (BAC) determination.
Daniela Cavagnino
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Aim
A newly designed static headspace autosampler connected to a dual GC-FID system was evaluated to offer forensic and clinical toxicology laboratories a fast, easy to use and reliable solution for routine blood alcohol analysis, combining high sample throughput to highly precise, accurate and defensible results.

Methods
The Thermo Scientific TriPlus™ 500 Headspace Autosampler was connected to a dual column/dual FID configuration of the Thermo Scientific Trace™ 1310 GC via a microfluidic 3-port connector. The GC was run in isothermal conditions to achieve maximum throughput along with ID confirmation. The main focus was to optimize the overall conditions to shorten the cycle time, while delivering the best area counts RSD%. Thanks to the TriPlus 500 HS new design, method set up is further simplified due to the reduced number of parameters required in the method. The Thermo Scientific Chromeleon™ CDS was used for instrument control and data acquisition, processing, and flexible data reporting. For targeted quantitative analysis, methanol, ethanol, acetone, isopropanol, acetonitrile, ethyl acetate, and 1-propanol (IS) stock standard at 10 g/dL in water (LC/MS grade) were prepared. Calibration standards over 5 concentrations (ranging from 0.01 to 0.2 g/dL) were prepared in water. A 1-propanol internal standard solution was prepared in water at 0.2 g/dL. Standards/blood samples or water blanks (500 µL) were transferred to 10 mL crimp headspace vials containing 60 µL 1-propanol (0.2 g/dL, IS). Target analyte separation was achieved with two capillary columns, a Thermo Scientific™ TraceGold™TG-ALC1 30 m x 0.32 mm I.D x 1.8 µm film and TraceGold™TG-ALC2 30 m x 0.32 mm I.D x 1.2 µm film.

To test method performance, whole blood control samples over five concentrations ranging from 0.02 to 0.3 g/dL, were analyzed and quantified against the mixed alcohol standards, with IS correction.

Results
• GC separation time < 3 min
• Ethanol linearity R2 > 0.999
• Ethanol area counts repeatability 0.8% (15 replicates, 0.1 g/dL std)
• No detectable ethanol carryover after high concentrated samples

Confident quantification of BAC in routine testing relies on stable analytes response in solvent standards and ultimately in blood samples. Repeatability of absolute peak area was tested in solvent standards as well as in blood samples, carrying out n=15 consecutive analyses of mixed alcohol standards at 0.04 and 0.1 g/dL in water and n=7 injections of whole blood certified control samples at 0.3 g/dL. Excellent %RSD between 0.7 and 3.2 % were obtained.

Discussion
The new static headspace autosampler design features a direct column connection to the HS valve manifold, significantly reducing the sample path to assure the best sample integrity during injection, while simplifying the method set up. A highly precise pressure control during sample transfer assures excellent area counts RSD%, increasing the confidence in the quantitative results. The new pneumatic control embedded in the headspace autosampler also assures a continuous purging of the sample path, eliminating possible carryover after high concentrated samples and therefore, the risk of possible false positive results. The highly inert and reliable microfluidic 3-port connector delivered improved GC connectivity without impacting on peak resolution, peak asymmetry values and assuring reproducible splitting between two columns. High throughput analysis is possible, aided by overlapped headspace incubation cycles and unattended sequences of up to 240 samples.

Conclusions
A newly designed Static Headspace Autosampler was evaluated in conjunction with a dual column/dual FID GC system for routine blood alcohol content determination with an optimized method for increased productivity, extended unattended operations and ease of use.

The proposed solution delivers short cycle time, improved RSD% and sample integrity during injection to offer the required data confidence and defensibility.

Optional barcode and auditing capabilities of Chromeleon CDS also allow operation in a regulated environment with the necessary data quality and traceability.
Abstract ID 131
Urinary concentrations of EtG and EtS after repeated ingestion of ethanol.

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Aim
Urinary concentrations and detection times for ethyl glucuronide (EtG) and ethyl sulphate (EtS) are previously studied, but no studies have investigated how repeated ingestions of ethanol affect the excretion pattern. The aim of this study was to investigate details in concentrations of EtG and EtS in urine after experimental repeated dosing of ethanol.

Methods
Thirty three subjects ingested a first dose of 0.51 gram ethanol per kilo body weight during the first 60 minutes of the study, and then a second dose of 0.25 g/kg (low dose group), 0.51 g/kg (medium dose group) or 0.85 g/kg body weight (high dose group) 120 minutes after start of the study. Urine samples were collected for seven hours after intake. All samples were analysed for EtG and EtS using a ultra high performance liquid chromatography tandem mass spectrometry method that was validated according to “Validation of new methods” by Frank T. Peters, Olaf H. Drummer and Frank Musshoff (Forensic Science International 165 (2007) 216–224).

Results
The results showed Cmax values for EtG of 9.02 mg/L (range 4.61-14.69), 13.71 mg/L (7.41-29.38) and 29.53 mg/L (14.44-29.78) in the low, moderate and high dose group, respectively. For EtS, the Cmax values were 3.08 mg/L (1.36-5.05), 3.50 mg/L (2.63-6.64) and 5.20 mg/L (4.73-6.99) in the low dose, medium dose and high dose group, respectively. The concentrations (Cmax) of EtS were significantly lower than the concentrations of EtG (p<0.001) and the Tmax for EtS appeared significantly earlier than the Tmax for EtG (p<0.001). The median Tmax for EtS was 270 minutes (range 240-300) in the low dose group, 300 minutes (range 240-360) in the medium dose group and 360 minutes (range 300-420) in the high dose group. From the concentration-time curves, the second intake of ethanol was evident for the medium and high dose groups, but not for the low dose group. Figure 1 shows the individual concentrations of EtG in the medium dose Group.

Discussion
Conclusions
In conclusion, this study showed the details in urinary concentrations of EtG and EtS after repeated dosing of ethanol. We found that EtG and EtS appeared in urine simultaneously, but EtG peaked later and at higher concentrations compared to EtS. Also, EtG showed a clearer dose-response relation compared to EtS. The two separate ingestions were both evident from the concentration-time curves when the last dose was sufficiently high.
Abstract ID 184
Quetiapine abuse in city of Adana, Turkey.
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Aim
The abuse of psychoactive drugs used for therapeutic purposes is becoming widespread at the present time. The case reports and data of poison centres associated with the potential for abuse or misuse of quetiapine, which is included in second-generation antipsychotic drugs, by oral, intranasal, and intravenous administration has indicated that quetiapine abuse is a common phenomenon in the last decade. The use of the drug in the treatment of psychiatric disorders such as bipolar disorder and schizophrenia is approved by the US Food and Drug Administration (FDA). It can also be used in the treatment of mood disorders, anxiety and major depression, alcohol and drug addiction in the clinic. Intentionally, quetiapine abuse may result in various undesirable behavioural changes, including anxiolysis, hypnosis and euphoria. The fact remains that the drug is not included controlled substances in Turkey and its drug abuse potential is often ignored.

In many studies, quetiapine has also been reported to be simultaneously abused with other illegal drugs, including sympathomimetic such as cocaine, and amphetamines. The frequency of use of quetiapine in these cases has attracted our attention while drug testing was performed in the biological samples of drug of abuse cases due to use of illegal drug. In this study, we aimed to present the rates of quetiapine usage in the blood samples of 4293 patients who came to our Forensic Toxicology Laboratory.

Methods
4293 drug of abuse cases related to quetiapine positive were evaluated retrospectively as a result of illicit drug testing was performed by LC-MS/MS methods on the cases that brought to Forensic Toxicology Laboratory in Faculty of Medicine of Cukurova University by law enforcement agencies. The all of the cases were drug abuser or seller. Quantification in only blood samples was performed using validated method in routine laboratory analysis. The urine samples were performed qualitative analysis. The cases were asked whether they used any prescription drugs when taking biological samples. The therapeutic drug users were excluded in this study.

Results
According to our results, quetiapine was positive in 50 of 4293 cases (1.16%). All of the positive quetiapine cases were male subjects. The other demographic information of the cases is not included in the files. While quetiapine was used alone in 11 of 50 cases with quetiapine positivity, quetiapine was used simultaneously with one or more group of illicit drugs in the remaining 39 cases. Quetiapine was most frequently detected in 10 of the cases with the opiate group drugs. The use of quetiapine alone with cannabis was found in 8 cases. The range of concentrations measured in blood was 0.46 to 411.0 ng/mL (mean: 56.4 ng/mL). This concentration range was not exceeding the therapeutic concentration (40 to 600 ng/mL).

Discussion
Many regular prescription drugs with circular issued by Turkish Ministry of Health in 2013, as a result of abuse and were followed by Turkey Pharmaceuticals and Medical Devices Agency but quetiapine still have not been included controlled substances. In the period of July 2005 - July 2016, the European Medicines Agency (EMA) received 209571 adverse drug reaction reports on quetiapine, and in 18112 reports on abuse / misuse / dependence / withdrawal corresponding to 884 patients and 8.64% of all adverse drug reactions reports have been written for quetiapine.

Conclusions
In this study conducted in Adana province, the most common use of cannabis and quetiapine was determined in cases with abuse of illicit substances. The monitoring of quetiapine is preventing drug abuse especially in prone population such as illicit drug abusers and addicts. Forensic Toxicology Laboratories play an important role in taking necessary prevention related drug abuse by sharing these data with related Turkish Government Agencies.
Abstract ID 208
N-ethylpentylone induced conditioned place preference in rats.

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Aim
N-ethylpentylone (NEP) is one of the most seized synthetic cathinones in the world was recently and temporarily placed in Schedule I by Drug Enforcement Administration, U.S. Department of Justice (effective during 8/31/2018-8/31/2020). However, little information is available about its addiction and toxicity. The aim of this study was to assess the addiction potential of NEP.

Methods
Conditioned place preference (CPP) model was conducted. The CPP test box comprised three plexiglass chambers connected by doorways that could be closed by removable guillotine doors. The two end chambers (32×30×50 cm) had black and white walls and floor, respectively. The central chamber (12×30×50 cm) had gray walls and floor. The CPP experiment consisted of 3 phases: the pre-test, the conditioning, and the CPP test. 1) The pre-test (day 3): after habituation for two days, SD rats were introduced into the central chamber of the CPP box with free access to all three chambers for 15 min, to get their baseline level of preference for time spent in each of the end chamber. Only rats that did not show an initial significant preference for both end chambers were kept in the further experiment (the time spent in each end chamber was not greater than 650 s, unbiased CPP). The end chamber in which rats spent less time was chosen as the “drug paired chamber”, and the other end chamber was used as the “saline-paired chamber”. 2) The conditioning (days 4-11): On days 4, 6, 8 and 10, rats were intraperitoneal injected with NEP (2, 5, 10, 20, 50 mg/kg) once daily on alternate days and then confined to the white chamber (“drug paired chamber”) for 40 min, with saline as the negative control and methamphetamine (METH, 5 mg/kg) as the positive control. On days 5, 7, 9 and 11, all rats were intraperitoneal injected with saline, and then confined to the black chamber (“saline-paired chamber”) for 40 min. 3) The CPP test (day 12): the rats were introduced into the CPP apparatus with free access to all three chambers for 15 min. The time rats spent in each end chamber was recorded by a digital video recorder installed above the CPP box.

Results
Rats administrated with NEP (5, 10, 20, 50 mg/kg) spent significantly more time in the drug-paired chamber during the CPP test, compared to the pre-test (p <0.01 or < 0.001). With the increase of NEP dosage, the preference score (CPP score) increased, which was expressed by the time spent in the drug-paired chamber minus the time spent in the saline-paired chamber. 5 mg/kg of NEP and METH showed no statistical difference in inducing CPP.

Discussion
CPP in rats was chosen based on the fact that CPP is a well-established and widely used model for assessing the addictive properties of drugs. NEP at low dose (5mg/kg) could induce CPP of rats and generate reward effects, which was partially dose-dependent. The addictive effect of NEP was smaller than that of MDPV, which could induce CPP behavior in rats in the dose range of 1.0-3.2 mg/kg. However, the addictive effect of NEP was significantly stronger than that of mephedrone, which required a dose of up to 30 mg/kg to induce CPP behavior in rats.

Conclusions
NEP could induce CPP in rats, and the lowest effective dose was 5mg/kg.
Abstract ID 219
Blood concentrations of designer benzodiazepines: Findings in forensic cases in Norway.

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Aim
In recent years, novel benzodiazepines have become available on the recreational drug market. Being sold to circumvent legislation, these compounds are usually referred to as designer benzodiazepines and are classified as a group of new psychoactive substances (NPS), although some of them were first synthesized many years ago as potential pharmaceutical drug candidates. Their use is increasing in many countries, but data concerning blood concentrations appear scarce, making interpretation of concentrations very difficult. Our laboratory analyzes blood samples from drugged drivers and other criminal offenders. In cases where etizolam or phenazepam, or one of the newer designer benzodiazepines clonazolam, diclazepam, flubromazepam, and flubromazolam is the only drug detected, the conclusion of the clinical test of impairment (CTI) can be used to investigate the relationship between concentration and degree of impairment. The aim of this study is to report blood concentrations of the aforementioned benzodiazepines and to investigate the relationship between blood concentrations and the degree of impairment that may follow.

Methods
The data included in this study are from blood samples collected from suspected drugged drivers and other drug offenders. In conjunction with apprehension, a CTI is performed by a physician, including single tests and a conclusion regarding impairment. Due to legal restrictions, only the conclusion was available in the present study. The blood samples were analyzed for designer benzodiazepines (clonazolam, diclazepam, flubromazepam, and flubromazolam) as well as etizolam and phenazepam, by ultra-high-performance liquid chromatography-tandem mass spectrometry using an Acquity UPLC with a Premier or a Xevo TQ-S tandem-quadrupole mass spectrometer in multiple-reaction monitoring mode (MRM) from Waters. Separation was achieved by gradient elution with either a mobile phase consisting of ammonium formate pH 5 and acetonitrile on a BEH C18-column (2.1x100 mm, 1.7 µm, Waters) or a mobile phase consisting of ammonium bicarbonate buffer pH 8 and methanol on a BEH Phenyl-column (2.1x100 mm, 1.7 µm, Waters). Positive cases from between June 1st 2016 and October 1st 2018 were included. Blood concentrations and the conclusion regarding level of impairment are reported.

Results
The presented six benzodiazepines were detected in 327 cases during the study period. The number of cases and the median (range) concentrations were as follows: clonazolam (n=21) 0.004 mg/L (0.002-0.053), diclazepam (n=189) 0.011 mg/L (0.002-0.246), etizolam (n=14) 0.069 mg/L (0.015-0.302), flubromazepam (n=5) 0.037 mg/L (0.007-0.700), flubromazolam (n=17) 0.008 mg/L (0.0004-0.036), and phenazepam (n=86) 0.026 mg/L (0.002-0.853). In 13 of the cases, a designer benzodiazepine was the only drug detected. The physician concluded with mild, moderate or considerable impairment in nine of the 13 cases. Most of the concentrations in these cases were relatively similar to or higher than the median reported concentrations. Among the remaining 314 cases, the most frequent other drugs detected were amphetamine, THC, and clonazepam.

Discussion
Conclusions
The presented blood concentrations can be helpful with the interpretation of cases involving one or more of these six benzodiazepines. The results indicate that concentrations commonly observed in forensic cases are associated with impairment.
Abstract ID 259
Development of high-sensitivity method to determination of LSD and 2-oxo-3-hydroxy-LSD in oral fluid by liquid chromatography-tandem mass spectrometry.

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Aim
Oral fluid is an excellent specimen in field sample collections (e.g. DUI/D, electronic music festivals context, etc.), as its collection is not invasive, it is more acceptable to the volunteers, and it does not require specific place or specialized professionals to perform the sample collection. 2-oxo-3-hydroxy-LSD (OH-LSD) is the major metabolite of the lysergic acid diethylamide (LSD) in urine, but no publications have described its presence in oral fluid. The aim of this study was to develop a sensitive method to quantify OH-LSD and LSD in oral fluid samples using liquid-liquid extraction followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The method was applied to the analysis of samples collected in the university and at electronic music parties.

Methods
Oral fluid samples were collected using the Quantisal™ (Immunalysis, USA). To 500 µL of sample (125 µL of oral fluid and 375 µL of Quantisal buffer) was added 20 µL of methanolic solution of LSD-d3 (10 ng/mL, internal standard) and 500 µL of saturated sodium tetraborate aqueous solution. The extraction was performed in the order of pg/mL.

Conclusions
A sensitive and reliable method to quantify LSD and its metabolite OH-LSD in oral fluid samples was fully validated and applied to analysis of authentic samples. OH-LSD is probably present at oral fluid without glucuronide conjugation, as enzymatic hydrolysis was not necessary for its detection. LSD metabolite concentration in oral fluid was much lower than the parent drug, requiring LOD and LOQ on the order of pg/mL.
Abstract ID  287
Prevalence of therapeutic drugs in blood of injured drivers in Italy. An epidemiological study.
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Aim
In Italy, the prevalence of drugs and medication in drivers has recently been assessed, but variables being measured across different studies vary significantly, and not all relevant psychoactive substances have been included in the toxicological assessment. This study is designed as a “classic” epidemiological study and aims to assess the prevalence of psychoactive medications used alone or in combination with alcohol and/or illicit drugs in injured drivers.

Methods
Samples of 1130 drivers involved in road accidents (injured drivers) in the area of Bologna, Emilia Romagna, Italy, in the period 2017-2018 were collected. The toxicological analyses were performed on whole blood taken as follows:
- Alcohol: GC-FID (Shimadzu QP 2010 Plus);
- Illicit drugs: CEDIA (ILab 650, Werfen) followed by confirmation analysis through GC-MS (Shimadzu QP 2010 Plus);
- Medications: (benzodiazepines and Z-drugs, BdZ; antipsychotics and antidepressants, AA; medical opioids, MO): LC/HRMS (Q Exactive™ Hybrid Quadrupole-Orbitrap™ Mass Spectrometer, ThermoFisher Scientific).

Fifty-two substances were assessed. The following cut-offs were used to identify positive samples:
- Alcohol: 0.5 g/L (Italian legislation).
- Illicit drugs: cut-offs proposed by international recommendations and widely adopted in the forensic setting.
- Medications: the lower therapeutic ranges are the minimal concentrations that are able to produce psychotropic effects, identified after the review of the existing literature.

The population was divided into groups, as follows. Gender; Age: ≤25, 26–35, 36–45, 46–60 and >60 year-old; Accident time: weekday (Monday–Thursday 8.00–22.00), weekendnight (Friday–Sunday 22.00–8.00). The positive results were compared with regard to the population groups identified. The Chi-square test or Fischer’s exact test, when appropriate, were used in order to assess differences among sub-groups. The significance level was set at the 5% value.

Results
The highest prevalence was found for alcohol (17.3%), followed by BdZ (7.3%), AA (7.2%), cocaine (3.4%), MO (tot=32; 3.1%). Fourteen different types of BdZ, 18 AA, and 3 MO were found. Among BdZ, diazepam/nordiazepam, delorazepam, clozapam and alprazolam were the most common. Sertraline, Paroxetine and Citalopram were the most common among AA. Only codeine, morphine and tramadol positive cases were present as MO. The presence of alcohol was significantly higher in male subjects (p=0.03). Night and weekend accidents showed a significantly higher prevalence of alcohol, both alone and combined with illicit drugs (p<0.01). The presence of illicit drugs was significantly higher in male subjects (p<0.01). Cocaine was found more frequently in the age range 26-35 (4.9%) and 36-45 (5.7%) (p=0.04). The presence of illicit drugs was significantly higher in night-time accidents (p<0.01). The presence of BDZ (p=0.03) and AA (p=0.03) was significantly higher in female drivers. An increasing trend at increasing age was found for AA (p<0.01). This was also partially confirmed for BdZ, in which the 26-35 y.o. had a higher prevalence than the group 36-45 y.o (p<0.01). The presence of medication were significantly higher during the week and in accidents occurring during the day (p<0.01).

Discussion
This study confirms that, besides alcohol and illicit drugs, medications are frequently detected in the blood of drivers involved in road accidents. If on one hand data for alcohol and illicit drugs partially overlaps with those reported in previous studies, the prevalence of BDZ was much higher. Furthermore, we found a high prevalence of AA, which are rarely investigated in epidemiological studies performed on drivers (no Italian data exist), but may cause impairment of the ability to drive, especially when taken in combination with alcohol or other drugs. Despite their well-known odd ratio for injury or death as a result of a car crash while under the influence, they have been added to the “core list” of substances studied by the DRUID only by few countries. The prevalence of MO (codeine, tramadol and methadone) does not consistently differ from previous Italian epidemiological studies performed in Italy and in other countries. As for the correlation between the groups and the toxicological results, we observed that the prevalence of BdZ and AA: • is higher in female drivers; • increases with age, being the highest after 60 year-old; • does not follow the same trend observed for alcohol and illicit drugs, since the number of medications-positive drivers is higher during the day and during the week.

Conclusions
The use of some class of medications (BdZ-AA) is mainly observed in female subjects and older people, and does not follow the same weekly trend observed for alcohol and other illicit drugs. We observed that illicit drugs and alcohol are commonly taken with medications and/or other drugs (cocaine was found alone only in 50% of cases). This may be an additional problem, since per se cut-offs proposed in most countries were established basing on the impairing effect of the drug alone, without considering the synergic effect with other drugs, which seems to be increasingly frequent in the population of injured drivers. Since the DRUID working group already addressed this problem, its relevance could and should be taken into consideration.
Abstract ID 291

Is the non-linear formation of EtG caused by differences in first pass metabolism of ethanol?

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Aim
A positive non-linear relation between dose of ethanol ingested and the area under the curve (AUC) for ethyl glucuronide (EtG) in urine is previously observed. However, the relation between AUC of ethanol and AUC of EtG in urine was linear. The relation between both doses and AUC of ethanol and the AUC for EtG in blood is not previously published and this study aimed to investigate this relationship.

Methods
10 healthy volunteers ingested 0.5 gram ethanol per kilo body weight (low dose) in one occasion and 1.0 gram ethanol per kilo body weight (high dose) in the next occasion after an overnight fast. Blood samples were collected at 1.5, 3.5, 5.5, 8.5, 11.5 and 24 h after start of drinking and analysed for ethanol and EtG. Ethanol was analysed using an enzymatic alcohol dehydrogenase method and EtG was analysed using a previously published ultra-performance liquid chromatography-tandem mass spectrometry method. The relation between both doses and AUC of ethanol and the AUC for EtG is studied using the non-parametric paired samples Wilcoxon signed rank test. EtG and ethanol concentrations are measured in mg/L and g/kg, respectively and for AUC calculations, the concentrations are multiplied with hours.

Results
There was a significant higher median ratio between blood AUC for EtG and dose of ethanol in the high dose (8.99, range 7.37-10.94) compared to the low dose (5.02, range 4.25-6.15) group (p=0.005). The median ratio between the AUC for EtG and AUC for ethanol was actually significantly higher in the low dose (1.77, range 1.51-2.24) compared to the high dose (1.67, range 1.30-2.02) group (p=0.005), although values are quite similar. There was also a significantly higher median ratio between AUC for ethanol and the dose of ethanol in the high dose (5.64, range 4.21-6.25) compared to the low dose (2.67, range 2.11-3.99) group (p=0.005).

Discussion

Conclusions
The ratio between the AUC for EtG in blood and dose of ethanol is higher after intake of 1.0 g/kg than 0.5 g/kg. This pattern is however not seen when AUC for EtG is compared to AUC for ethanol. This study therefore supports that the percentage of ethanol converted to EtG is not increasing when the doses increase. An explanation for the positive non-linear relation previously observed between the dose of ethanol ingested and amount of EtG formed may be a relative higher first pass metabolism of ethanol at lower doses.
Abstract ID  299

Serum Ethyl glucuronide as a marker of recent abuse of ethanol in DUI cases - the Italian current situation.

Angelica Capomassi

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Aim

One purpose of the study is to relate alcohol elimination kinetics with that of EtG formation in the time between 4-5h after intake (e.g. accident time) in order to support the hypothesis that the subject at the time of the accident was in a state of drunkenness and thus provide a more realistic picture of the alteration state related to alcohol intake and not rely only on data obtained from the withdrawal in E.R. that often happens several hours later accident.

Secondly, to evaluate usefulness of serum and urinary EtG to detect relapses in subjects under treatment for chronic alcoholism.

Methods

100 alcoholics in care on which we evaluated BAC, serum and urinary EtG.

30 volunteers who ingested standard doses of alcohol and performed 4 blood withdrawals starting from 1h after ingestion, 1h apart on which we measured alcohol and EtG.

Results

In volunteers values of BAC were decreasing and EtG values increasing.

Blood and urine samples from alcoholics detected relapses, with serum EtG values up to 5.8 mg/L Urine EtG up to 7.4 mg/L, while BAC was undetectable.

Discussion

Use and abuse of alcohol is widespread between young and old and can lead to dependence. The intake of alcohol before driving is a relatively common behavior among the population but driving under the influence (DUI) of alcohol is punished by Italian Highway Code with fines, suspension of the driving license and arrest. In Italy, three categories of increasing fines are investigated, to date back the BAC the subject had while driving and possibly caused a car accident. Furthermore, there may be delays in execution of the withdrawal under chain of custody (requested by prosecutor) in E.R. Knowing that alcohol elimination rate is about 0.20 g/L/h, time to make the withdrawal is very narrow. For many years alternative solutions had been investigated, to date back the BAC the subject had while driving.

Currently in Italy to prove the DUI, the BAC measured at the time of the withdrawal in E.R. when the subject is conducted by the police is considered.

This value does not represent the “under influence” state the subject had at the time of driving and possibly caused a car accident. Furthermore, there may be delays in execution of the withdrawal under chain of custody (requested by prosecutor) in E.R. Knowing that alcohol elimination rate is about 0.20 g/L/h, time to make the withdrawal is very narrow. For many years alternative solutions had been investigated, to date back the BAC the subject had while driving.

Many markers are known but their main issue is that these markers are not specific to ethanol and mostly detect a time window too far from the recent intake needed to punish DUI of alcohol.

EtG in blood is detectable from 1h after alcohol intake and up to 14h with peak at 3.5-5.5h making it useful for the detection of a recent intake of alcohol when not supported by the BAC detected.

In urine EtG can be found from 1h and up to 90h after, making it useful to monitor in the short term a person in care at addiction centers, and for toxicological monitoring of alcohol abstinence in drivers, armed forces, driving licenses, etc. In forensic DUI cases it would be useful using BAC, combined with serum EtG and urinary EtG to have a complete picture of the alteration state of the subject and possibly identify a potential alcoholic (with urinary EtG) and address him to proper addiction centers.

EtG in serum allows to detect a state of recent intoxication even when alcohol in blood has already been metabolized or it’s under the threshold limit for driving (in Italy 0.5 g/L). It is even more useful performing two withdrawals 1h distant from each other and measuring both the BAC and the serum EtG. So we see that a very recent assumption (within 4h, e.g. hip-flask defence) will show increasing EtG values, while a recent intake will show decreasing values of EtG.

Conclusions

In DUI cases it would be appropriate using BAC and serum EtG values of at least two consecutive withdrawals to assess whether the subject had been drinking before the accident, indicatively what time, and what might have been his BAC at the time of accident, relying on volunteers’ data. Alcoholic’s serum and urinary EtG have proved effective in identifying those who have experienced a relapse but their BAC was negative.
Abstract ID 306
A portrait of drugs and driving prior to cannabis legalization in Québec (Canada).

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Aim
In 2008, the International Drug Evaluation and Classification Program (DECP) was introduced in Canada by a modification to the Criminal Code. Under this program, arrested drivers are subjected to a twelve-step standardized process by a drug recognition expert (DRE), including psychophysical coordination tests and the collection of a biological sample, typically urine. Since then, the program has grown in Canada and constituted the most used investigative path for driving under the influence of drugs (DUID) cases up until further legislative changes accompanying recreational cannabis legalization were enacted in October 2018.

This study aims at drawing a portrait of this legislatively regulated period under the DECP and highlight trends in drug consumption in the province of Québec (Canada).

Methods
All DECP cases analyzed by the Québec forensic toxicology laboratory between January 2014 and December 2018 were included in the present study. Although the precise analytical workflow applied on the biological samples varied throughout the years, all results were confirmed by a second analysis. From 2014 to 2017 only, immunological analysis (AxSYM®, Abbott Laboratories) was carried out prior to solid phase extraction (SPE) on Oasis®HLB cartridges (Waters) and general unknown screening using a GC-MS/NPD (Agilent Technologies) complemented with a targeted analysis by LC-MS/MS (4000 QTRAP®, AB Sciex). After July 2017, samples were extracted by protein precipitation followed by targeted screening on a 5500 QTRAP® LC-MS/MS (Sciex). γ-hydroxybutyric acid (GHB) presence was confirmed either via enzymatic assay (MGC-240, Thermo Scientific) or derivatization followed by GC-MS analysis (Agilent). If deemed necessary (~6%), for example due to the lack of toxicological findings compatible with the signs and symptoms described by the DRE, the SPE treated sample was sent to general unknown screening by GC-MS/NPD where new psychoactive substances (NPS) could be detected.

Data for all cases were compiled in a Microsoft Access® database, including gender and age of the suspect; date, time and location of the arrest; detected drugs and opinion of the DRE as to which category of drugs caused impairment (central nervous system (CNS) depressants, inhalants, dissociative anesthetics, cannabis, CNS stimulants, hallucinogens and narcotic analgesics).

Results
A total of 2 983 DECP cases were analyzed from January 2014 to December 2018. In almost all cases (99%), urine was the biological matrix collected by the DRE. Male individuals were overrepresented in these DUID arrests (79% of arrests vs 52% of driving license holders), as were younger individuals (59% of individuals arrested were between 16 and 34 years old vs 37% of driving license holders).

Toxicological findings showed that CNS stimulants were more prevalent than CNS depressants, with 72% versus 61% of cases. The most prevalent drug findings in 2018 were methamphetamines (56% of cases), cannabis (47%), cocaine (31%) and GHB (20%). This repartition was overall constant over the years. Polydrug abuse, defined as cases where two drugs or more, excluding ethanol or non-active substances and metabolites, were detected, is common in DUID (>75% of cases).

Negative cases are a rare occurrence, with 66 cases only (2%). Typically, these cases were not only submitted to the in-house general unknown screening processes, but some were also sent to an external laboratory for analysis such as high resolution mass spectrometry.

NPS have been detected in a relatively limited number of DECP cases (24, <1%) and included furanyl fentanyl, acetyl fentanyl, fluoroamphetamine, gidazepam, flubromazolam and etizolam.

Discussion
Urine has so far dominated DECP cases as the biological matrix of choice, most likely due to its ease of collection. This is unfortunate from a toxicological standpoint since only past use can be inferred. However, the most recent changes to the Canadian Criminal Code lower the required standard for blood collection and should favour its acquisition, with or without a DRE evaluation.

The drug findings described in this study demonstrate once more how cultural differences can breed separate toxicological ecosystems. Indeed, amid a North American opioids’ crisis, CNS stimulants are still more prevalent in DUID cases than depressants and narcotic analgesics are, a finding which is at odds with the rest of the Canadian provinces. The surprisingly high prevalence of GHB is another example of this cultural phenomenon, mirrored in the near absence of GHB seizures in other provinces, whereas it is the fifth most identified substance in Québec.

Conclusions
The collection of data on DECP cases has allowed to draw a portrait of driving under the influence in the province of Québec (Canada) over the four years before the legalization of recreational cannabis in October 2018. Already, cannabis was the second most prevalent drug, found in 47% of all cases.

However, comparison with post-legalization data will require a careful analysis of the biases at hand, since the number of DREs trained will increase and changes to the Criminal Code will allow easier access to blood samples. The number of DUID cases analyzed by the forensic toxicology laboratories is thus bound to increase.
Abstract ID 329
Driving under the influence on cannabis in Portugal (drug level, impairment and result interpretation). A 8 years retrospective study.
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Aim
In Portugal, cannabis (Δ9-Tetrahydrocannabinol) remains the most commonly used drug among drivers. To assess the impairment level of cannabis on driving is a fairly complex issue since there is no threshold value defined in Portuguese legislation (impairment for criminal offences). The purpose of this study is to show the prevalence and concentration of cannabis (Δ9-tetrahydrocannabinol) among drivers involved in traffic accidents samples for the last 8 years (2011-2018). Another relevant aspect that will be addressed in this study will be the association between cannabis and alcohol since it is widely described in the scientific literature that the consumption of both substances significantly increases the impairment level on driving skills.

Methods
6644 blood samples were collected from drivers involved in traffic accidents. Blood samples were taken by medical professionals in hospitals as part of their routine tasks and preserved refrigerated in polypropylene tubes containing potassium fluoride and EDTA until analysis. The average time between the incidente and blood sample collection was 2 hours. Cannabis (D9-Tetrahydrocannabinol) was analised by a fast ultra-performance liquid chromatographic with eletrospy ionization tandem mass spectrometry (UPLC-ESI-MS/MS) and alcohol was analysed by a headspace gas chromatography with flame-ionization detection (HS-GC–FID). LOD for D9-Tetrahydrocannabinol and alcohol were 0.5 ng/mL and 0.1 g/L respectively. In addition to cannabis other drugs of abuse (opiates, cocaine and amphetamines) were also analyzed by GC/MS.

Results
819 samples (12.9%) were positive for cannabis (Δ9-tetrahydrocannabinol). Among the positive samples 94.6% (n=775) come from men (16 - 65 years old) and 5.4% (n=44) come from women (18 - 63 years old). The average concentration was 2.7 ng/mL and the median was 1.8 ng/mL (range 0.5 - 27 ng/mL).

Discussion
It was possible to observe an increase in the number of positive drivers for cannabis over the years (10.6% in 2011 and 14.5% in 2018). In 51% of the cannabis positive samples (n=419) was also possible to detect the presence of alcohol (range 0.2 – 3.8 g/L). It was also possible to observe that the median concentration of THC in drivers who not have alcohol (1.6 ng/mL) is slightly lower than the median of the drivers who consume cannabis and alcohol simultaneously (1.9 ng/mL).

Conclusions
The collected data from drivers involved in traffic accidents in the last 8 years shows an increase of the use of this substance and a high association of alcohol and cannabis. Since there is no threshold, level in the Portuguese legislation the impairment of cannabis on drivers remains undetermined.
Abstract ID 346
Psychoactive substances, their metabolites and drugs occurring in the blood of drivers investigated at the Institute of Forensics Genetics in Bydgoszcz in 2018.

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Aim
Recently, there has been a significant increase in the interest in new psychoactive substances (NPS). New illegal substances appear on the black market, which are also found among drivers. Increased market availability of NPS results in an increase of road hazards, as these substances are the cause of numerous accidents and become a serious threat to the life and health of both users and other drivers. The widespread immunochemical testing model used as a screening method has become insufficient. Modern instrumental methods characterized by high sensitivity, specificity and rapid analysis are subjected to thorough validation in terms of as many analytes as possible with a minimum duration of the analysis to detect and determine the psychoactive substances and their metabolites.

Methods
A LC-MS/MS system (2x Exion LC AC Pump, Exion LC Degaser, Exion AC Autosampler, Exion LC Column Oven coupled with a Sciex 5500 QTRAP mass spectrometer operated in ESI mode) and certified standards of individual analytes belonging to various chemical groups was used for the validation of the analytical method developed for 549 analytes according to the SWGTOX criteria. The samples were extracted by liquid-liquid extraction. Gradient elution was performed on separation column (Kinetex C18 100 x 3 mm, 2.6 μm). The buffered methanol/water mobile phase system was used. The MRM pair monitoring mode was used for qualitative and quantitative analyzes of each of the analyzed substances. A total of 1220 blood samples from traffic participants were subjected to statistical analysis.

Results
Limit of quantitation, BIAS (the maximum acceptable BIAS is ±20% at each concentration), precision (the % CV shall not exceed 20% at each concentration), reproducibility, specificity and matrix impact were determined during the validation. A positive result of the validation carried out according to the SWGTOX criteria was obtained for 521 analytes. The Institute of Forensics Genetics in Bydgoszcz analyzed 1220 blood samples from drivers: 1179 men and 41 women. Only 366 of the tested samples did not contain any of the analyzed substances. Due to the qualification of drivers mostly based on a positive result of screening tests, the presence of amphetamines and THC was found most frequently in the blood of drivers. In many cases, in which the initial indication was obtained in road tests, the presence of new psychoactive compounds was found: synthetic cannabinoids, cathinones and opioids. In case of drivers diagnosed with impaired driving ability, the presence of GHB, 3,4-MDMA, THC, THCCOOH, lidocaine, alpha-ethylaminopentiophenone, amphetamine, methadone, alpha-hydroxymidazolam, paracetamol, 7-aminonclonazepam, clonazepam, morphine, methamphetamin, 4-CMC, N-propylamphetamine, tramadol, propafenone, fluoxetine, sertraline, sildenafil, clomipramine, lorazepam, norclomipramine, benzoylcegonine, codeine, zolpidem, diphenhydramine, estazolam, diazepam, desmethyldiazepam, N-propyl-2Al, ketamine, norketamine, cocaine, N-ethylpentylone, 4-hydroxydiazolam, alprazolam, N,N-dimethylpentylone, carbamazepine, cocaine, clomipramine, 2-MMC, 3-MMC, 4-MMC, 4-methylcathinone, methylhexanamine, alpha-ethylaminohexanophenone, benzylamid, 5-fluoro-ADB, 5-fluoro-NPB-22, 4-methyl-PHP, 4-chloro-alpha-PVP, 4-CEC as well as the coexistence of many psychoactive compounds was found.

Discussion
The obtained validation parameters allowed the introduction of the developed method for routine analysis. Research on samples of drivers shows how much of them contains psychoactive substances. As described above, only 366 of the 1220 samples tested did not contain any of the substances analyzed. In 315 of the positive samples only one substance was detected. Amphetamine was determined in 444 samples and THC in 259. This shows, that large percentage of drivers use these psychoactive substances. However, in other samples, other psychoactive substances available on the black market appeared. The number of samples in which no substance has been confirmed suggests the further development of the ours method in order to be able to develop and detect NPS in the blood of road traffic participants.

Conclusions
The method developed for the determination of psychoactive drugs, their metabolites and pharmaceuticals based on LC-MS/MS meets the conditions of validation according to SWGTOX and was introduced to routine analysis enabling a more complete monitoring of the impairment of psychomotoric abilities of drivers resulting from the intake of psychoactive compounds and seems to be the optimal solution in the era of more common recreational use of new psychoactive drugs and drug abuse.
Abstract ID 351
Determination of ethanol in blood samples collected in the central region of Portugal in scope of the Road Code (2017-2018).
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Aim
Among the expertise requested to the National Institute of Legal Medicine and Forensic Sciences (INMLCF, I.P.), toxicological tests to evaluate the state of influence by alcohol and psychotropic substances, in particular with regard to road driving supervision, constitute the great majority. According to article 152º of the Road Code (approved by the Decree-Law no. 114/94, of May 3), the evaluation of states of influence by alcohol or psychotropic substances should be done in drivers, pedestrians, whenever they are involved in traffic accidents and people who intend to start driving. Article 156º of the same decree adds that all drivers and pedestrians involved in a traffic accident must, whenever their state of health allows it, undergo an exhaled breath test, and that when it has not been possible to carry out this examination, they must be taken to an official health establishment to do a blood test. With regard to the supervision of driving under the influence of psychotropic substances, article 157º stipulates that all drivers and persons intending to start driving must be examined for the detection of psychotropic substances when there is evidence that they are under the influence of such substances.

Methods
Considering that the examinations for alcohol and psychotropic substances research in blood are performed at the INMLCF, IP, and in order to obtain a reality perspective for the central region of Portugal for this type of exams, a survey of the number of expertises requested by the National Road Safety Authority to the Forensic Chemistry and Toxicology Service (SQTF) of the INMLCF, IP - Center Branch, between 2017 and 2018 for alcohol and / or psychotropic substances, was made. For the examinations related to alcohol testing, the respective framework (e.g., gender, age) and results were analyzed. The analytical results for the determination of ethanol were obtained in the SQTF using analytical procedures that include gas chromatography, according to article 6º of law no. 18/2007, of May 17, in order to research and, if necessary, quantify the presence of this substance in blood samples.

Results
Comparing the two years under study in the same period, the number of required blood analysis only differed in 7 (1054 in 2017 and 1048 in 2018). Similarities were also found relatively to the age group and gender most submitted to the alcohol test (≥50 years, male gender). In both years under study, the vast majority of drivers who underwent alcohol analysis were involved in accidents (799 and 634 in 2017 and 2018, respectively), as were pedestrians with values of 97 and 65. Finally, the great majority of alcohol analysis in 2017 and 2018, respectively and regarding the same period were: negative (295 and 296) or positive between 1.2 and 2 g/L (299 and 318). In decrescent order, the remaining BAC values were: higher than 2 g/L (188 and 185), between 0.8 and 1.2 g/L (128 and 126), between 0.5 and 0.8 g/L (86 and 68), and, lastly, between 0 and 0.5 (55 and 53).

Discussion
No significant differences were found between the two years under study, regarding the number of blood tests and their motive (e.g. driver, accident participant), to the blood alcohol concentration range predominantly found in this type of exams and, finally, to the gender and age group most submitted to blood analysis to determine its BAC.

Conclusions
The present study allows to conclude that there is a trend over the two years that may or may not be maintained over the time. For this reason, in a future analysis, it would be interesting to study the evolution observed in several consecutive years, in order to obtain a broader study and can thus verify if the trend remains in a larger sample.
Abstract ID 353
Alcohol and illicit drug use by victims with severe trauma admitted to the biggest hospital in Latin America in Sao Paulo, Brazil
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Aim
Investigate the use of alcohol and illicit drugs (cocaine, cannabis and amphetamine-type stimulants) by patients with severe trauma hospitalized in the Clinics Hospital of the Faculty of Medicine of University of Sao Paulo.

Methods
Patients were recruited from August/2017 to October/2018. Trained nurses collected blood samples at the moment they were admitted to the emergency room, using Vacutainer® glass tubes with fluoride to prevent degradation. Patients who were admitted more than 6 hours after injury were excluded, as well as, those who were younger than 18 years old. We included only patients who were severely injured, were hospitalized for more than twelve hours. Informed consent was provided after a few hours or days after admission, once patients were stabilized and were able to talk. Cases that patients remain unconscious or confused for more than ten days, informed consent was provided by patient’s family, as well as, answers to questionnaire. Blood samples were analyzed at Oslo University Hospital (Norway) by UHPLC-MS/MS. The following cutoffs were utilized: Δ9-THC: 1 ng/mL; Cocaine: 10 ng/mL; Benzoylecgonine: 50 ng/mL; Anhydroecgonine methyl ester (crack cocaine marker): 10 ng/mL; Amphetamine: 20 ng/mL; Methamphetamine: 20 ng/mL; MDMA: 20 ng/mL; Alcohol: 0.2 g/L.

Results
During the study period, 438 patients were attended on the emergency room. Those that did not fulfill the inclusion criteria (i.e.: under 18 years old, non-trauma injuries and transference to other hospitals) had no blood samples collected, therefore, 190 blood samples were collected, 31 patients died before interview and two (1%) refused to participate. Finally, 157 patients were included on the study. The majority of the patients was injured in traffic crash (n=110, 70%), followed by fall-injured patients (n=27, 17.2%) and victims of interpersonal violence (n=20, 12.7%). The average age was 36.5 years and most of them were men (82.2%). Regarding the toxicological findings, 46.5% samples (n=73) tested positive for a psychoactive substance. Alcohol was the most prevalent (38.2%), followed by cocaine (16%) and cannabis (5.7%). No amphetamine-type stimulant was detected. Among the traffic crash victims, 53.6% were drivers/riders, dominated by motorcyclists (76.3%).

Discussion

Conclusions
Nearly half of the severely injured patients tested positive for psychoactive substances. Alcohol was most frequently detected, followed by cocaine and cannabis. Almost three quarters of the patients were injured in traffic crashes.
Abstract ID 377
Advancing Forensic DUID Screening with Mass Spectrometry
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Aim
Quadrupole Time-of-flight mass spectrometry (QTOF-MS) provides high-resolution, accurate-mass data for full-scan information of both precursor ion and all product ions. This is an ideal approach for forensic toxicology screening where unknown compounds in complex biological samples must be identified from information-rich data sets.

In this technical note, a comprehensive drug screening workflow for the analysis of forensic Driving Under the Influence of Drugs (DUID) blood samples is described. This novel DUID method was successfully used to obtain retention times and MS/MS spectra necessary to build a targeted analysis workflow for 60 forensic compounds. The adaptation of QTOF-MS technology enabled identification of multiple number of the targeted compounds present in authentic forensic DUID case samples in comparison to immunoassay-based screening.

Methods
Control whole blood samples were spiked with a stock standard solution mixture and extracted for LC-MS screening to determine retention times. Forensic DUID case samples and control whole blood samples were extracted by using a protein precipitation prior to being reconstituted in mobile phase for analysis.

Analytes were chromatographically separated at 30°C using a Phenomenex Kinetex phenyl-hexyl (50 x 2.1 mm, 2.6 µm) column. Mobile phase was ammonium acetate in water and ammonium acetate in acetonitrile plus formic acid, 500 µL/min flow rate. Mass spectrometric detection was conducted on a X500R QTOF System operating in positive electrospray mode with information dependent acquisition (IDA) MS/MS method and negative electrospray mode with multiple reaction monitoring (MRM) MS/MS method, respectively. Samples were evaluated against four main confidence criteria weighted as follows: mass error (15%), retention time (30%), isotope ratio difference (5%), and library score (50%) for all compounds. These criteria were used to generate a combined score. The processing criteria for positive identification of an analyte in a sample required all four main confidence criteria to pass.

Results
The spiked control whole blood samples were successfully used to obtain retention times and MS/MS quality spectra to build a data analysis processing method and high resolution spectral libraries for the 60 target analytes.

Two different acquisition strategies were employed to streamline the screening workflow. For all positive ionizable compounds IDA-MS/MS was chosen as the acquisition mode, as it enabled the easy collection of precursor ions, and multiple dependent MS/MS scans on several of the most abundant precursor/candidate ions. For the remaining target compounds that favor negative electrospray ionization, MRM was used as the acquisition strategy due to the additional selectivity it provides monitoring unique parent ions. This detection mode was found to be beneficial as it enhanced compound identification at the LOD by performing MS/MS spectral library matching.

Discussion
The implementation of a robust method development process resulted in high combined scores for all compounds based on the four main confidence criteria defined in the processing method. Additional quantification strategies were implemented by setting an analyte concentration threshold based on the LODs to minimize false positives and/or false negative hits, which resulted in LODs in the sub ng/mL range, mass errors less than 2 ppm and MS/MS scores over 90%. In addition, average (n=9) combined scores for all target compounds were calculated using the control blood samples spiked at the LOD analyzed over the course of 3 days. Inter-day reproducibility resulted in %RSDs ranging between 1-10% for the target analytes, showing the robustness and reproducibility of the overall workflow.

Conclusions
Overall, the developed QTOF-MS screening approach was compared to the traditional immunoassay-based screening method since it is often considered the method of choice for screening samples. In addition to requiring less forensic blood sample volume, the current QTOF-MS enabled the identification of multiple number of target compounds present in authentic forensic DUID case samples that were not detected in the immunoassay-based screening method. The use of the QTOF-MS screening approach was also shown to greatly reduced false positives when compared to immunoassay-based screening methods.
Abstract ID 418
Laura Huppertz
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Aim
The benzodiazepine gidazepam was developed in the former Soviet Union in the early 1990s. It is a registered prescription drug in Ukraine, used as daytime tranquilizer in neurotic, psychotic, and anxiety conditions, as short-term treatment for sleep disorders or emotional lability, and to reduce the severity of withdrawal symptoms in alcoholism therapy. The drug is available as 20 or 50 mg preparation for intravenous, oral or sublingual administration. Gidazepam has a broad therapeutic range, acting as an effective anxiolytic combined with activating and antidepressant properties and no sedative effects at low doses. The benzodiazepine has a high bioavailability and acts as pro-drug for its main active metabolite desalkyl-gidazepam (bromo-nordazepam). The elimination half-life of gidazepam and desalkyl-gidazepam is relatively long (86 h). With most articles in the literature being in Russian or Ukrainian, access to data on the pharmacodynamics, pharmacokinetics, and toxicity of gidazepam is limited. Since gidazepam is not approved as prescription drug in Canada, it is not routinely screened for.

Here, we present the case of a 31 year-old woman found motionless in the driver's seat of a vehicle with the engine running, arrested for driving under the influence of drugs. A Standardized Field Sobriety Test (SFST), followed by a drug recognition evaluation (DRE) and urine sampling were performed. In the woman's car and purse, police found and seized several drugs - including a blister containing two out of ten tablets with Cyrillic lettering printed on the aluminum-foiled back - and a make-up bag containing about 40 syringes, three spoons, and nine small plastic bags.

Methods
The content of the seized blister pack was analyzed by GC-MS, and the urine sample was screened using a targeted LC-MS method for 110 frequently encountered drugs in Montréal, Canada. An aliquot of the urine sample and one of the pills from the blister pack were sent to Freiburg, Germany and analysed using LC-QTOF MS and LC-MS/MS. Gidazepam, obtained as prescription drug from a Ukrainian pharmacy was used as authentic standard for confirmation.

Results
According to the DRE officers report the subject showed ptosis and persistent drowsiness. She had great difficulties standing up, continually losing her balance. When she was asked to walk straight ahead, she was unable to do so. She staggered and leaned against the wall in order to lift her leg. During the interrogation she fell asleep several times, requiring the officer to wake her up. Vertical nystagmus, reaction to light and the ability of the subject’s eyes to converge were not evaluated due to the pronounced ptosis and drowsiness. Heart beat and blood pressure were 56 bpm (mean), and 90/62 mmHg, respectively, indicating a light bradycardia and hypotension. The woman’s body temperature was slightly lower than normal, and her muscle tone was rigid, causing her difficulties to relax her arms.

Methadone was detected in the urine sample by LC-MS. The GC-MS analysis of the pill from the blister pack revealed the presence of an unknown, benzodiazepine-like compound. Regarding the DRE results and the almost empty blister pack with the Cyrillic lettering for ‘Gidazepam’, intoxication with this uncommon benzodiazepine was suspected. However, it was not possible to identify the substance in the tablet or urine sample, as there was no commercial standard available for confirmation, and gidazepam was not included in any of the in-house or online databases accessed (Cayman, NIST and SWGDRUG) at the time of the analysis.

LC-MS/MS and LC-QTOF MS analysis both detected gidazepam next to lorazepam. Additionally, diphenhydramine, methadone, EDDP, fluconazole, levetiracetam, nicotine and metabolites, and caffeine and metabolites were detected in the performed LC-QTOF MS screening. In-silico metabolite prediction for gidazepam was performed and the following metabolites of gidazepam were identified based on accurate mass, fragment information, mass defect filtering and isotope-cluster analysis: desalkyl-gidazepam, hydroxyl-desalkyl-gidazepam, gidazepam-carboxylate (loss of the hydrazine moiety), and gidazepam-carboxylic ester (loss of the hydrazine moiety and intramolecular esterification). These findings confirmed the initially suspected gidazepam intoxication.

Discussion
To our knowledge, this is the first case of gidazepam intoxication reported outside of Ukraine or Russia. Due to the scarcity of information on gidazepam available to forensic toxicologists, result interpretation of cases involving this drug can be challenging. Moreover, in the case presented here, the driver additionally consumed lorazepam and methadone, a narcotic analgesics which can exhibit similar effects to those of benzodiazepines. Thus, the subject’s impairment may be caused by either of the drugs detected.

Conclusions
The presence of the drugs in urine and the DRE results suggest drug uptake, but ultimately, the individual contribution of gidazepam and the severity of impairment could have only been determined with confidence if a blood sample would have been available.
Abstract ID 445
Evaluation of ethanol levels in traffic accidents in Turkey with the scope of legal limits.

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Aim
Traffic accidents are a major public safety problem for Turkey like other developing countries. The number of road traffic deaths continues the climb, reaching a high of 1.35 million in 2016 over the world according to World Health Organisation (WHO) report. Alcohol has been shown to be the most common cause of traffic accidents in many countries. Alcohol decreases significantly the driving skills and capabilities due to the influence on concentration, alertness and the reflexes of individuals, including drivers, pedestrians and vehicle passengers. Under the Turkish road traffic regulations, for professional drivers such as taxis, minibuses, buses, alcohol limit is 21 mg/dl while 50 mg/dl for other drivers. Izmir, Turkey’s third largest city, is in third place in terms of overall traffic accidents in our country. The aim of this study is to evaluate the alcohol levels in blood samples of the cases who applied due to traffic accidents in Ege University within the scope of national and international legal limits.

Methods
Blood samples obtained from 4266 traffic accident cases between 2016-2017 were delivered to the Addiction Toxicology Laboratory with an established chain of custody. Ethyl alcohol analysis was performed by using enzymatic immunoassay method (CEDIA). Cases above the detection limit of 10 mg/dl were reported as positive. The data obtained from the retrospective examination of hospital automation system were analyzed statistically (SPSS 25.0).

Results
Traffic accident cases were 21.3% (n=4266) of all cases (n=20058) who applied for alcohol analysis. The mean age of the all cases was 32.4 ± 12.4 and 70.4% of the cases were male. Considering the causes of traffic accidents, 49.5% of them were vehicle passenger, 26.7% motor vehicle and motorcycle driver, 19.5 % pedestrians, %1.5 bicycle driver, %0.2 of them were truck or van passenger. According to the age classification by taking into account the official status of the driver’s license, 6.8% of cases were under age of 18. Ethyl alcohol was found to be positive in 9.6% (n=411) of the traffic accident cases. In ethyl alcohol positive traffic accident cases, 83.7% (n=344) were male and 16.3% (n=67) were female and 33.1% (n = 136) of them were in the 25-34 age range. The minimum age of the alcohol positive cases was 12 years. The most positive cases were applied in Spring. 4.1% (n = 175) of the cases, ethyl alcohol concentration was found to be 100-199 mg/dl. There were significant differences in the alcohol positive traffic accident cases according to the years. An increase was observed in 2017 compared to 2016 (p<0.05). %87.6 (n=360) of the positive cases were found to be above the legal limit 50 mg/dl. 57.7% (n = 237) of the alcohol positive traffic accident cases were vehicle passenger and 27.2% (n = 112) were motor vehicle and motorcycle driver.

Discussion
In a study conducted by WHO, 25 of 191 countries found that the legal limit of blood alcohol in traffic was zero while 53 of them was reported to be 50 mg/dl. Considering the risk of alcohol limits in the scope of the studies, in countries including Turkey with a legal limit of 50 mg/dl and above, they are considered to pose a risk to traffic even within legal limits. According to the our study’s results, it was found that number of alcohol positive cases are increased by years. It has been observed that alcohol positive vehicle passengers are particularly under the risk at traffic. In our study, it was determined that the cases who had a traffic accident and alcohol detected were the individuals belonging to the young population. The study in the United States showed that in some states the age of alcohol use death decreased by 10-15% as a result of increasing the age of alcohol use from 18 to 21 years. In our country, the age of obtaining a driver’s license and the age of purchasing alcohol are the same (18 years).

Conclusions
The restructuring of the laws in Turkey and the deterrence of penalties, the frequent and legal limit of inspections as zero will be an important step in preventing traffic accidents. It is foreseen that the risk of traffic accidents may be decreased by increasing the age of buying alcohol.
Abstract ID 452
Illicit psychoactive drug use among Brazilian truck drivers. Henrique Bombana

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Aim
Verify the use of illicit drugs (amphetamine, cocaine and cannabis) by truck drivers through toxicological analysis in urine and oral fluid.

Methods
We have collected 492 urine and oral fluid samples during 2016 from drivers passing through Sao Paulo’s federal highways who took part in health preventive actions carried out by the Federal Highway Police, entitled “Health Commands on the Roads”. We recorded socio-demographic and occupational data by using a structured questionnaire in order to study any association with the toxicological results. Oral fluid was collected with Quantisal™ device and urine in polyethylene bottles. Both had previously been labeled with a number linking the anonymous sample to the questionnaire. Samples were screened within ten days after collection by immunoassay methods for amphetamine, cocaine and cannabis using “Multi-Drogas One Step” (Inlab, Brazil) for urine and Enzyme-Linked Immunosorbent Assay (ELISA—Immunalysis, Pomona, CA, USA) for oral fluid samples. The considered cut-off values for urine and oral fluid samples, respectively, were: ∆9-THC — 50 and 4 ng/mL; cocaine — 300 and 20 ng/mL; amphetamine — 1000 and 50 ng/mL. The presence of drugs was confirmed by gas chromatography and ultra performance liquid chromatography coupled to mass spectrometer for urine and oral fluid respectively, during 2018. The cut-off values used for the confirmation step were: THC-COOH—15 ng/mL; benzoylecgonine — 150 ng/mL; amphetamine — 200 ng/mL for urine analysis and ∆9-THC — 1 ng/mL; cocaine/benzoylecgonine — 10 ng/mL; amphetamine — 25 ng/mL for oral fluid analysis.

Results
Of the collected samples, twenty-eight oral fluid samples (5.7%) and forty-six urine samples (9.3%) presented a positive result for some of the psychoactive substances studied. Of these, the majority presented positive results for cocaine or its metabolite, benzoylecgonine, (20 for oral fluid and 25 for urine), followed by amphetamine (9 for oral fluid and 20 for urine) and THC (∆9-tetrahydrocannabinol) or its metabolite, THC-COOH, (2 for oral fluid and 5 for urine). There were less positive samples in oral fluid matrix. Nevertheless, in the two types of biological matrices studied, there were more positive cases for cocaine and amphetamine.

Discussion
Some Brazilian studies have already pointed out the use of illicit drugs by truck drivers and here we have confirmed that they are still consuming stimulants to be able to withstand their exhausting work journeys. The similar findings in the two different matrices shows that not only truck drivers have consumed, but also could be under effect of some substance in the moment of sample collection. Use of stimulants and cannabis plays a significant role for occurrence of road traffic crash. Therefore, it is important that efficient public policies can be implemented in order to make Brazilian roads safer.

Conclusions
Cocaine or its metabolite was the most detected substance in oral fluid and urine, followed by amphetamine and cannabis or its metabolite.
Abstract ID 480
An Experimental Study of Diazepam and Its Metabolites Pharmacokinetics in Oral Fluid Following Signal Oral Dose.
Lele Wang
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Aim
1. Background: Driving under the influence of drugs was met in China, its annual occurrence was apparently accelerating, and traffic accident was boosting accordingly. Saliva testing for drugs offers significant advantages over blood and urine as a test matrix. Collection can be performed in almost any location, with less embarrassment and under directly observed conditions. It has been widely used in the field of medicines testing as well as the identification of drug abuse or drug-driving.

2. Objective: The study was supported by the National Science and Technology special project work (2015FY11400) and National Key R&D Project (2017YEC0803504, 2018YFC0807403). It aimed to found some bio-markers in saliva to identify the ingestion of diazepam through a basic pharmacokinetic information of diazepam and its metabolites in saliva, and then testify for relevant cases.

Methods
1. Methods: A total of twenty eight healthy volunteers given a signal oral dose with 5mg of diazepam were included by consent of Committee of medical ethics of Shanxi medical university (2014087). Saliva were collected at prior (zero), 1 h, 2 h, 4 h, 8 h, 12 h, 24 h and 2 d, 3 d, 6 d, 12 d, 15 d post-consumption time-points from each participant with a commercial available collector. All samples were extracted with solid phase extraction and analyzed with high performance liquid chromatography tandem mass spectrometry with positive MRM model. The pharmacokinetic analysis was performed using a compartmental method with a software of 3P97.

Results
1. Results: Only diazepam and its main metabolite, nordazepam, could be detected in the saliva of volunteers, and the window detection was within six days to more than fifteen days. The pharmacokinetics of diazepam in saliva met a two compartment model, t1/2k01, t1/2k12, t1/2k10, Cmax, Tmax were 0.7±1 h, 31.4±68.5h, 12.1±11.6h, 4.1±4.1 ng/mL, 1.1±0.8h; The pharmacokinetics of nordazepam in saliva met an one-compartment model, t1/2k01, t1/2k10, Cmax, Tmax were 41.5±44.8h, 282.3±365.5h, 0.5±0.2ng/mL, 80.3±59.2h.

Discussion
1. Discussion: The analytical method developed in this study has proved to be sensitive and accurate for the analysis of diazepam and its metabolites in blood. The dosage (5 mg) used in the study was close to the therapeutic dosage of the drug (5-10 mg), suggesting that the method might be suitable for monitoring driving under the influence of diazepam. However, we just found a metabolite, nordazepam, in the saliva of volunteers, and no oral fluid results were positive for OZ, OG and TG after intake of D. Because D’s excretion into oral fluid is mainly caused by simple diffusion through salivary glands, the molecules excreted into saliva need to be unbound, non-ionised and lipid soluble, therefore, OG and TG could not passe into oral fluid easily. While for OZ and TZ, there are different results at present. The lack of OZ in oral fluid after administration of a signal oral dose has been previously reported by Vidar Temte eal. However, Laloup et al. once analyzed oxazepam in the oral fluid from three volunteers. Nordal KM eal. reported that metabolites identified in oral fluid after ingestion of diazepam include TZ, ND and OZ, but there was a difference in the concentration of different analytes in different volunteers. The authors concluded that racial differences is the possible reason. Besides, after the consumption of 5mg diazepam, the detection window of diazepam and nordazepam ranged from an hour to more than 15d, and t1/2k10 of diazepam and nordazepam was 12.1h±11.1, 282.3h±365.5, they provided a longer time limit to identify diazepam ingestion as well. This discovery suggests that diazepam and nordazepam can act as specific markers of diazepam consumption for at least 15 days and their relative concentrations can be used to eliminate the possibility of co-administration of diazepam with other benzodiazepines such as temazepam and oxazepam. However, they both had an individual differences as DiGregorio et al. reported. They reported that concentrations in the range of 1-6 ng/mL over an 8-hour period after an acute oral dose of 10 mg of diazepam, while Hallstrom and Lader reported mean concentrations in the range of 0–15 ng/mL over a 96-hour period in 6 subjects after the same dose. Accordingly, it should be considered comprehensively dealing with such cases.

Conclusions
1. Conclusion: The above data suggest that nordazepam was the only metabolite could be detected in saliva of Chinese with diazepam history. It had a longer detection window, and could be used as a bio-marker in saliva of diazepam ingestion, while they both had an individual differences in the pharmacokinetics. It should be considered comprehensively when it was applied to such cases.
Abstract ID 500
Study on the Pharmacokinetics of Alcohol and Its Metabolites in Chinese.
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Aim
1. Background: In most countries, alcohol consumption distributions have been shown to possess universal features. Driving under the influence of alcohol increases the risk of crash as well as the severity of crash-related injuries. Efforts to reduce alcohol-impaired driving have included implementing laws regarding blood alcohol concentration (BAC) limit when driving, and detecting BAC is the main way to identify and quantify alcohol. However, detection widow of alcohol is generally 6-8h. In order to improve efficiency, metabolites of alcohol, especially for ethyl glucuronide (EtG) and ethyl ester (EtS), have been studied to prove alcohol ingestion. The pharmacokinetics of alcohol and its metabolites was useful for the identification of alcohol driving, while they are almost vacant in Chinese. Moreover, metabolites in urine with longer detection window have been reported. The study aimed to investigate the pharmacokinetics of alcohol and its metabolites (EtG and EtS) in whole blood and urine of Chinese.

2. Objective: To observe the pharmacokinetics of alcohol and its metabolites (EtG and EtS) in whole blood and urine of Chinese.

Methods
Methods: A total of twenty eight healthy volunteers given 40% alcohol (0.72g/kg) orally. Five milliliter blood were collected at prior (zero), 0.5 h, 1.5 h, 2 h, 3 h, 5 h, 8 h, 12 h, 24 h, 36 h, 48 h, 120 h post-consumption time-points from elbow vein of each participant, ten milliliter urine were collected at prior (zero), 0.5 h, 1.5 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 12 h, 15 h, 24 h, 36 h, 48 h, 120 h post-consumption time-points. All samples were extracted with liquid-liquid extraction and analyzed with head space gas chromatography (alcohol) and high performance liquid chromatography tandem mass spectrometry (metabolites). The pharmacokinetic analysis was performed using a compartmental method with a software of 3P97.

Results
Results: After an oral dose of alcohol, the detection window of alcohol in blood and urine was similar, it was at 8h after alcohol ingestion that we could not detect alcohol in both blood and urine of almost all volunteers. For EtG and EtS, they showed a longer detection window in urine (36-48h) than in blood (24-36h). The pharmacokinetics of alcohol and its metabolites (EtG and EtS) in both blood and urine met a one-compartment model, t1/2k01, t1/2k10, Cmax, Tmax of alcohol, EtG and EtS in blood were 19±0.45h, 1.16±0.30h, 46.66±11.11mg/100mL, 1.43±0.47h; 2.02±0.39h, 2.05±0.30h, 0.28±0.10μg/mL, 3.58±0.21h; 1.95±0.36h, 1.95±0.36h, 0.15±0.04μg/mL, 2.81±0.53h; t1/2k01, t1/2k10, Cmax, Tmax of alcohol, EtG and EtS in urine were 1.10±0.38h, 1.10±0.38h, 34.26±10.47mg/mL, 2.05±0.42h; 2.53±0.83h, 2.60±0.82h, 85.55±65.72μg/mL, 3.43±1.43h; 1.83±0.88h, 2.35±1.37h, 22.19±16.73μg/mL, 7.82±11.51h. However, the study reported the result having a remarkable difference among different individuals.

Discussion
Discussion: It is the first study reported pharmacokinetics of alcohol and its metabolites (EtG and EtS) in blood and urine of Chinese. The pharmacokinetics of alcohol in human has been reported a one-compartment model, which is consistent with the study, it was metabolized quickly within 8h after drinking. EtG and EtS, as the direct metabolites of alcohol, have been widely accepted bio-markers in forensic and clinical settings, each of these drinking indicators remains positive in serum and urine for a characteristic time spectrum after the cessation of ethanol intake--EtG and EtS in urine up to 7 days. The study showed a detection window of EtG and EtS in urine was 36-48h. They are relatively narrower but longer than what in blood. In this study, EtG and EtS in urine are still good indicators due to the other pharmacokinetics parameters to identify alcohol ingestion within a certain time. However, a high inter-individual variability in the pharmacokinetics parameters of alcohol and its metabolites has been noticed. There exists many elements like race, dietary, metabolism, amount, kind and duration of drinking can influence the pharmacokinetics of alcohol and its metabolites. Therefore, much attention should be attached to the research and utilization of such cases.

Conclusions
Conclusion: The pharmacokinetics of alcohol and its metabolites (EtG and EtS) in blood and urine of Chinese were established simultaneously, which was firstly reported. Although they showed some difference among different individuals, they would be useful for the clinical alcohol test and the diagnosis of alcohol. In the field of forensic science, it can also provide a basis for the identification of alcohol.
Abstract ID 516
Decrease of FAEE and EtG in hair by shampoo and water.
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Aim
Ethyl glucuronide (EtG) and fatty acid ethyl esters (FAEE) were widely used as long term markers for the detection of alcohol biomarkers in hair for indicating abstinence, e.g. for driver's license revocation. Recently, washout effects were detected for EtG when treated by swimming pool water or special detox shampooing. We now investigated washout effects for FAEE besides EtG by a similar procedure.

Methods
For this purpose, head hair - obtained from a regular hair cut - of four different subjects with known regular alcohol consumption (between 20 g alcohol per week and 120 g alcohol per day) was treated with water or with "High Voltage Detox Folli‐Cleanse" shampoo in cold or warm water for 0 to 7.5 hours. Each hair sample was mixed manually. To achieve a homogenous hair sample, several small portions of approximately 20 mg of hair from one volunteer were picked out and recombined to yield aliquots of 200 mg which were weighed into 5 mL glass vials. This resulted in seven homogeneous 200 mg aliquots per person. Then these hair samples were incubated. For the shampoo-water-incubation, 0.5 mL of shampoo was added to 4 mL of deionized water. Incubation of one aliquot with 4.5 mL deionized water was used as control. In addition, one aliquot of each hair sample was measured without incubation to determine the initial concentrations of EtG and FAEE. For incubation with shampoo-water at room temperature, samples were placed in an overhead shaker, operated at the lowest speed for 2.5, 5 and 7.5 hours, and for incubation with water, for 5 and 7.5 hours, respectively. For the incubation at 45 °C, samples with shampoo-water were placed in a tempered water bath. After incubation all samples were washed five times by manually shaking with 3 mL deionized water for approximately 20 seconds to remove any shampoo residues. Finally, the hair samples were dried overnight at room temperature and split into two aliquots, one for EtG and one for FAEE determination. EtG and FAEE were quantified with fully validated methods based on LC-MS/MS and HS–SPME–GC–MS, respectively.

Results
The measured alcohol biomarker concentrations ranged from below 5 (LOD) to 96 pg/mg for EtG, and from 0.21 to 2.1 ng/mg for ethyl palmitate. We found decreases for every condition. As expected from previous experiments, the samples incubated and shaken with shampoo-water showed a decrease of EtG between 43 and 53 % in total. The highest decrease per time occurred within the first 2.5 hour period (35 – 40 %). Ethyl palmitate showed a decrease of 15 to 38 % after 7.5 h. The washing with shampoo at 45 °C for 7.5 hours showed a drop in EtG concentration below the cut-off concentration of 5 pg/mg (decrease approx. 100 %) and the ethyl palmitate concentration for each sample was reduced by approx. 70 % after incubation in warm shampoo-water. The sample of a volunteer, who only drank 20 g alcohol per week, was artificially coloured and did not contain any EtG but 0.21 ng/mg ethyl palmitate before incubation.

Discussion
Preanalytical stability of samples is an important issue in forensic toxicology. Furthermore adulterations of hair samples due to extensive washing, chemical treatment or heat are important. Moreover naturally occurring washout effects or degradation processes due to excessive perspiration (sauna, other causes), contact with warm water or shampoo-water, swimming or environmental influences (e.g. UV light) can cause a decrease in analyte concentrations in hair. With this work we could show that these effects should be considered and further investigated. However, the performed experiments have model character, not reflecting "real life hair treatment". On the other hand, it is remarkable that despite these environmental influences and possible attempts for adulteration of hair, in most forensic cases there is still a link between alcohol consumption and the results of hair analysis for alcohol markers.

Conclusions
When using warm water with this special ("detox") shampoo, the decrease was very severe for both, EtG and FAEE. For incubations at room temperature with water or shampoo-water the effects were less severe. The results show that hair analysis may not provide unbiased results: individual washing and shampooing routines may have the potential to influence the individual alcohol biomarker concentration in hair.
Simultaneously Identify and Quantify Synthetic Cathinones in Urine Samples by Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry.

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Aim
Recently, New Psychoactive Substances (NPS) have become a serious drug problem all over the world. According to the Taiwan Food and Drug Administration, which states that as of 2018, there are already 144 NPS have emerged. In Taiwan, urine test for drugs such as Amphetamines, ketamine, and morphine have been researched in many related kinds of literature, but there are few studies about NPS. The aim of this study was to establish a high-resolution liquid chromatography/mass spectrometry-based analytical method to simultaneously identify and quantify synthetic cathinones in urine samples. Also applied the established method to examine sixty cases of drug abuse in Taiwan.

Methods
Specimens were extracted using liquid liquid extraction with 5mL ethyl acetate for twice. After vibration for 10min and centrifugation with 5200rpm for 10min, the organic phase was transferred to a clean glass extraction tube and then evaporated to near dryness using a gentle stream of nitrogen gas at 45oC bath. The tube was reconstructed with 200μL of mobile phase for Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry (LC-QTOF-MS) analysis. Analytes were ionized via positive electrospray ionization, followed by All Ion MS/MS (AIM) mode. With the accurate molecular weight (bias within ±10%) and isotope peak ratio of mass spectra of precursor ion and qualified at least one product ion with the coelution of the precursor in AIM mode, we could confirm and quantify the substances in urine samples without misjudgments. The method have been validated to simultaneously qualify and quantify the common drugs abused in Taiwan, include mephedrone, chloromethcathinone (CMC), chloroethcathinone (CEC), MEAPP, methylenone, ethylone, butylylene, pentylone, dibutylone, N-ethylpentylone, MDPV, alpha-PVP and Cl-alpha-PVP. Also applied the robust method to search and confirm the metabolites which might be specific markers of synthetic cathinones in urine samples.

Results
In method validation, the linear dynamic range (weighting factor of 1/X) was as follows (mephedrone-d3 or MDPV-d8 as the internal standard): mephedrone, dibutylone, MEAPP and Cl-alpha-PVP (2.5–1000 ng/mL); CMC, CEC, methylenone, ethylone, butylylene, pentylone, MDPV and alpha-PVP (5–1000 ng/mL); N-ethylpentylone (50–1000 ng/mL). The coefficient of determination are more than 0.9968. Recovery of LLE at 10, 50, and 500 ng/mL were in the range of 86.1–120.2%, within-day and between-day accuracy (bias %) were in the range of -18.8–18.2% and precision (CV %) were in the range of 0.2–11.1% for all compounds. Sixty urine samples collected from suspected drug abusers by the police department in Taiwan were analyzed using the established method. Of these, 38 samples were positive for mephedrone (6–10154 ng/mL), 13 for dibutylone (6–8226 ng/mL), 18 for MEAPP (5–20937 ng/mL), 4 for Cl-alpha-PVP (4–141 ng/mL), 1 for CMC (235 ng/mL), 10 for CEC (7–1366 ng/mL), 12 for mephedrone (14–10154 ng/mL), 5 for ethylone (1–220 ng/mL), 12 for butylone (5–2284 ng/mL), 5 for pentylone (5–3407 ng/mL), 2 for MDPV (7–16 ng/mL), 1 for alpha-PVP (32 ng/mL) and 14 for N-ethylpentylone (51–14264 ng/mL).

Discussion
The results revealed the presence of synthetic cathinones abuse seriously in Taiwan. Approximately 68.3% of samples were positive for an NPS in combination with other drugs of abuse, especially ketamine (mostly consumed for recreational drug use), which indicates the popularity of NPS among teenagers. Because of special coffee packages (coffee powder mixed with multiple drugs), chocolate, or jelly are generally mixed with synthetic cathinones or another NPS, nimetazepam/phenazepam, or some other medicines, indicates a tendency for users to abuse multiple drugs simultaneously and causes lethal crisis. Furthermore, for N-alkylated and 3,4-methylenedioxy-N-alkylated synthetic cathinones, not only did N-dealkylation and beta-ketone reduce metabolism but metabolites of benzylc oxidation and aliphatic hydroxylation were also identified in real samples after acid hydrolysis. The markers might offer a method for confirming the abuse of NPS.

Conclusions
The number of abusers of synthetic cathinones has increased remarkably worldwide. Using high-resolution mass spectrometry has improved the specificity of targeted and nontargeted analysis, and the established method provides a faster approach for identifying and quantifying 13 types of synthetic cathinones in urine samples simultaneously. In addition, the accurate mass and secondary mass spectrum patterns of LC-QTOF-MS enable the confirmation of the metabolites of NPS and yield more information concerning drug abuse for more applications. In this study, the results reveal the trends of NPS abuse and provide a foundation for regulating these drugs to lessen the detrimental effects on society. However, additional NPS continue to be synthesized, and thus we remain dedicated to stay abreast of the latest NPS developments.
Abstract ID 16

Full Automated Forensic Routine Dried Blood Spot Screening for Workplace Testing.

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Aim
Increasing amounts of prescription and illicit drugs are driving clinical laboratories towards more cost effective, faster screening methods for workplace drug testing, roadside testing, rehabilitation programs and post-mortem investigations without compromising established false positive or negative detection rates. Therefore, increasing numbers of samples, each with a large panel of analytes, requires an improvement in sample throughput, achieved by shortening analysis time via a simplified sample preparation procedure. In this study we present a fully automated dried bloodspot (DBS) extraction procedure coupled to a LC-MS/MS-system for forensic and toxicology screening of drugs and their metabolites.

Methods
The DBS-MS 500 system (CAMAG, Switzerland) was coupled as a front end extraction device to a modular Nexera X2 UHPLC system from Shimadzu (Kyoto, Japan). Analysis was performed in positive multiple reaction monitoring (MRM) mode on ESI tandem mass spectrometry systems 8040 and 8060 (Shimadzu, Kyoto). Using a novel approach, the MRM Spectrum Mode was used to measure at least 5 MRM-transitions of each compound, generating a high degree of confidence for result identification. Data interpretation was performed by Labsolution Insight Screening software. Analytical standards were purchased from Lipomed (Switzerland). DBS cards (Ahlstrom TFN filter paper) were provided by CAMAG (Muttenz, Switzerland). Chromatography was performed on Shim-pack GIST (2.1 x 50 mm, 2 μm C18) (GL Science, Japan) and Kinetex (2.1 x 100 mm, 2.6μm, XB-C18) (Phenomenex, USA) analytical columns using a gradient of 10 mmol/L ammonium formate + 0.1% formic acid in water and methanol as mobile phases A and B.

Results
Full automation for the intended LCMS workflow of DBS cards was achieved by coupling the CAMAG DBS-MS 500 to the Shimadzu LCMS-system. The cards were photographed with a build-in camera and checked with image recognition software. The cards were then sprayed with internal standard (D5-alprazolam, D3-cocaine; 0.1 μg/ml in methanol) and extracted in a fully automated process. 20 μl of internal standard was sprayed in a homogenous layer onto each spot and dried for 20 sec. Samples were extracted with a volume of 22 μl and 50 μl/min flow rate with 70% methanol. Up to 500 cards with 4 samples each can be handled without any human interaction. Inadequate DBS samples were automatically excluded from analysis, based on their roundness, diameter and area. The total time from extraction to-result was 5 minutes per sample using a method with 28 drug target analytes. Chromatographic peaks were identified by screening against a spectral library containing more than 1200 forensic compounds.

The calibration levels were measured 6-fold on two different days to determine the inter-day and intra-day assay stability. The relative standard deviation of the internal standard was below 15 % for all target compounds when comparing data through all levels (5-1000 ng/mL). Inter-day variations were below 15% at the 25 ng/ml level, with the exception of diazepam (29.9%). Inter-day variations of this screening method were balanced by measuring a high and low quality control sample prior to sample analysis. All results were referenced against these values. The QC cards were prepared by spraying two calibrant solutions (100 ng/mL) for both basic and acidic target compounds onto 20 μl blank dried blood spots. For the QC sample, 10 μl was sprayed for the low, and 40 μl for the high QC sample, equivalent to a final extraction amount of 0.126 ng and 0.504 ng, respectively, when the plunger area of the DBS extraction unit (4 mm diameter) was taken into account.

Discussion
Through fully automated sample handling, increased sample throughput is achieved via a standardized workflow. This process reduces the cost per sample, increases reproducibility, and permits new methods of study design, in which a larger, broader population can be screened. This permits streamlining of the entire process, from sample extraction to results generation, as DBS samples are easier to transport than traditional liquid blood samples, permitting the use of equipped, centralised laboratories for the analytical work.

Conclusions
The process of DBS is minimally invasive, ensures post-sampling stability, permits shipment in standard envelope without biohazard classification and requires no sample cooling. Cards are received at the laboratory and processed in a fully automated system without any human interaction. The concept and benefits of DBS have been proven in several studies and will emerge in other markets such as clinical and pre-clinical pharmacology in the near future.
Abstract ID  64  
Study on distribution of cannabinoids in cannabis plants by desorption electrospray ionization mass spectrometry imaging (II).

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Aim  
Cannabis sativa L. is the most abused plant in the world. A total of 120 cannabinoids have been isolated from the cannabis plant, and some of them have psychoactivities. The major active cannabinoid of C. sativa is Δ9-tetrahydrocannabinol (Δ9-THC) and there are also non-psychoactive cannabinoids with several medicinal functions, such as cannabidiol (CBD). In the plant, Δ9-THC and CBD exist as their carboxylic acid forms, tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA), respectively. We investigated the contents of 11 cannabinoids in each part of the cannabis plant by the LC-Q-TOF-MS analysis and the distributions of THCA and/or CBDA by the desorption electrospray ionization (DESI)-Q- ion mobility separation (IMS)-TOF-MS analysis. Furthermore, we confirmed the surface aspect of the cannabis plant by the microscopic analysis.

Methods  
In this study, we examined three types of cannabis: a drug-type, a fiber-type and an “unknown-type” that strain characteristics may not be useful for the differentiation of the types of the cannabis plants.

For the LC-Q-TOF-MS analysis (TripleTOF 6600 system, SCIEX), the leaves, buds, bracts, seeds, stems and roots were used as samples. Each sample was sonicated in ethanol and centrifuged three times consecutively. The supernatants were combined, and the solution was subjected to a membrane filtration. The resulting solution was appropriately diluted and used for the measurement. The theoretical values of the protonated molecule ions of the 11 cannabinoids were monitored in the positive mode. A calibration curve was prepared by the internal standard method using the peak area ratio with an internal standard (Δ9-THC-d9). For the DESI-Q-IMS-TOF-MS analysis (DESI-Synapt G2-Si system, Waters), leaves, buds, bracts, seeds, stems and roots were used as samples without any pre-treatment. The sample was flattered and fixed to a slide glass and placed onto the 2D moving stage of the DESI source. The morphology of the cannabis plants was observed using the DESI-TOF-MS imaging analysis.

Discussion  
In this study, the morphological characteristics (such as shapes of leaves) of the “unknown-type” cannabis plant seemed to be the fiber-type. On the other hands, as a result of the quantitative analysis of the cannabinoids in the “unknown-type” plant using the LC-Q-TOF-MS, the composition of the cannabinoids was almost the same as the drug type. This result was consistent with that of the DESI-Q-IMS-TOF-MS analysis. The morphological characteristics may not be useful for the differentiation of the types of the cannabis plants.

Conclusions  
We investigated the contents and distributions of the cannabinoids of each part of the cannabis plants by the LC-Q-TOF-MS analysis and the DESI-Q-IMS-TOF-MS imaging analysis. Moreover, we confirmed the surface aspect of each part of the plants by the microscopic analysis. The results of these analyses were well consistent. The DESI-Q-TOF-MS imaging analysis will be useful to visually recognize the distribution of cannabinoids in each part of the cannabis plants.
Abstract ID 66
Quantification of clitidine in caps and stems of poisonous mushroom Paralepistopsis acromelalga by liquid chromatography–tandem mass spectrometry.
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Aim
Paralepistopsis acromelalga (former name: Clitocybe acromelalga, Japanese name: Dokusasako) is poisonous mushroom which belongs to Tricholomataceae family. A couple of accidental poisoning due to ingestion of P. acromelalga take place almost every year in Japan and South Korea. Among various compounds contained in the P. acromelalga mushrooms, clitidine is suspected as toxic component which brings various symptoms including erythromelalgia; marked swelling and redness accompanied by severe pain due to dysfunction of blood vessels which occurs at every terminal parts of the patients (e.g., extremities, nose and ear lobe)

In this study, simple and high-throughput analytical method for determining clitidine in Paralepistopsis acromelalga using hydrophilic interaction liquid chromatography tandem-mass-spectrometry (LC–MS/MS) was established, and applied to mushrooms obtained from 5 locations in Japan.

Methods
Clitidine was purified and crystallized from the collected wild mushrooms of Dokusasako (P. acromelalga) by co-author. Its purity was almost 100% with LC-MS analysis. Kainic acid to be used as internal standard (IS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other common chemicals used were of the highest purity commercially available. For determining clitidine in the mushrooms, simple procedure including dilution with methanol solution and filtering by cartridge was employed just before quantification by LC–MS/MS for high-throughput analysis.

A 0.1-g aliquot of P. acromelalga was crushed and diluted to 1:10,000 folds with 50% methanol aqueous solution. Then, the 1.0 mL aliquot was filtered by Captiva ND Lipids cartridge, following adding internal standard and appropriate target compound.

For LC separation, a TSKgel Amide-80 column (150 × 2.0 mm i.d., particle size 3.0 μm, Tosoh, Tokyo, Japan) was used. The LC conditions were: injection volume, 3.5 μL; flow rate, 0.25 mL/min; elution mode, gradient with 10 mM ammonium formate / 0.1% formic acid in distilled water (A) and acetonitrile (B) from 60% A/40% B to 100% B over 5 min, followed by isocratic elution with the initial solvent composition for 10 min. In this study, concentrations of clitidine in mushrooms were quantitated by the standard addition method. The calibration curves were made with 7 plots, which were determined in n = 3 at each point.

LC–MS/MS with electrospray ionization (ESI) was conducted on an Agilent 1200 LC-SL system connected to a 6460 Triple Quad LC/MS tandem MS instrument. The tandem MS condition were: interface, ESI mode; polarity, positive ion mode; ion source temperature, 320°C; ion source voltage, 500 V; quantification, selected reaction monitoring (SRM) mode using the peak area; ion transitions, m/z 271 → 139 for clitidine and m/z 214 → 168 for kainic acid (internal standard); fragmentor voltage and collision energy, 120 V and 9 eV for both compounds, respectively.

Results
Clitidine and internal standard could be analyzed by LC–MS/MS using our procedure, demonstrating sharp and well separated peaks in relatively short analytical time. Then, the present established method was also applied to analysis of fruit bodies of P. acromelalga, which were obtained from 5 different locations in Japan. Results on concentrations of clitidine in each stem and cap of P. acromelalga specimens tested showed that their concentrations were quite different not only between stems and caps in the strain, but also among locations; the concentrations of clitidine varied from 3.17 to 14.4 mg/g in caps, and 1.41 to 9.30 mg/g in stems, respectively.

Discussion
For all comparable mushroom samples, marked differentiation was observed in our measurements for concentrations of clitidine in stems and caps of P. acromelalga mushrooms examined. Concentrations of clitidine in caps were found to be higher than those in stems in the same strains tested. As for distribution of other toxic constituents in poisonous mushrooms, distribution of ibotenic acid and muscimol in Amanita muscaria mushrooms has been demonstrated to be well consistent with our results on clitidine in P. acromelalga mushrooms, in previous study. The amount and distribution of clitidine in P. acromelalga mushroom could be influenced by not only environments where the mushrooms were obtained from, but also differences among the strains; because we obtained a single mushroom from each location in this study, there are possibly remarkable differences in distributions and concentrations of clitidine among P. acromelalga mushrooms.

Conclusions
The literature describing analysis of toxic constituents in poisonous mushroom is still of the relatively limited number. In this study, we have established a detailed procedure for quantification of clitidine in P. acromelalga using LC–MS/MS, following dilution and filtration by a cartridge. This is the first report to present the detailed quantitative analysis of clitidine by MS and the distribution of clitidine in stems and caps of P. acromelalga. This analytical method for clitidine was thought to be useful in P. acromelalga poisoning cases to identify the causative toxic mushroom.
Detector of Synthetic Cannabinoid Metabolites Through Human Liver Microsome Incubation Followed by LC-ESI-Q/TOF Assisted by METABOLITE ID Software.

Sungill Suh

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Aim
Metabolite detection is critical for documenting the suspected drug consumption. For novel synthetic cannabinoids (SCs), however, there is not enough information about their metabolomics pathways and in many cases there exist no commercial reference metabolites for the field forensic laboratories. The aim of this study is to identify the metabolites of novel SCs using the pooled human liver microsome (pHLM) incubation followed by LC-ESI-Q/TOF high resolution mass spectrometry with a METABOLITE ID software.

Methods
ADB-FUBINACA and CUMYL-PEGACLONE were chosen as representatives of SCs. Metabolites were identified after the incubation with pHLM using LC-ESI-Q/TOF high resolution spectrometry in positive ion mode (compact QToF, Bruker). The metabolite predicting approach was enabled by the METABOLITE ID software (metabolitepredict 2.0 SR1 and metabolitedetect 2.0, SR1, Bruker).

The reaction mixture for ADB-FUBINACA included 78 μL of distilled water, 10 μL of 0.5 M phosphate buffer, 1 μL of NADPH regenerating solution B, 1 μL of ADB-FUBINACA (38.4 μg/mL in methanol and 5 μL pHLM (Xtreme 200 Human Liver Microsome – a pool of 200, 100 males/100 females characterized for 10 CYPs, FMO and 5 UGTs). After vortexing and centrifuzing, the reaction mixture was incubated at 37°C. The reaction was initiated by adding 5 μL of NADPH regenerating solution A and quenched with 100 μL of cold acetonitrile and stored at -80°C until analysis.

The reaction mixture for CUMYL-PEGACLONE included 58 μL of distilled water, 20 μL of 0.5 M phosphate buffer, 10 μL of Superoxide Dismutase (SOD), 5 μL of NADPH regenerating solution A, 1 μL of NADPH regenerating solution B and 5 μL of pHLM. After vortexing and centrifuzing, 1 μL of CUMYL-PEGACLONE (1000 μg/mL in methanol) was added and the reaction mixture was incubated at 37°C. The reaction was quenched with 100 μL of cold acetonitrile and stored at -80°C until analysis.

The metabolite separation was achieved on a Bruker Intensity solo C18 (100 2.1 mm, 1.8 μm). The full scan TOF-MS and the information-dependent acquisition MS/MS data were acquired. Already known or predicted metabolites were synthesized, three of which were the metabolites of ADB-FUBUINACA and two of which were those of CUMYL PEGACLONE, and also analyzed by LC-ESI-Q/TOF for the reference analysis.

Results
As for the ADB-FUBINACA, an amide hydrolysis metabolite (accurate mass m/z 384.1718, mass error 0.00 ppm) was identified through the pHLM incubation. However, the metabolite was not predicted by the METABOLITE ID software. The METABOLITE ID predicted the formation of an amide hydrolysis-dehydrogenated metabolite, which was detected from the pHLM incubation mixture with a low intensity.

In the case of CUMYL PEGACLONE, the pHLM incubation produced an aromatic hydroxylated metabolite (accurate mass m/z 389.2223, mass error 0.00 ppm) and an alkyl chain hydroxylated metabolite (accurate mass m/z 389.2226, mass error 0.77 ppm). Both of the mono-hydroxylated metabolites were also predicted by the METABOLITE ID software. The METABOLITE ID also predicted a dehydrogenated metabolite, which was detected from the pHLM incubation mixture with a low intensity.

Discussion
The pHLM incubation of ADB-FUBINACA produced low concentrations of metabolites and after 4 hours of incubation, ca. 47% of ADB-FUBINACA was not still metabolized. The metabolite concentrations were not increased after 4 to 16 hours of incubation, thus the effective incubation time was set for 4 hours for ADB-FUBINACA. The pHLM metabolizing rate of ADB-FUBINACA was slow and numerous metabolites were produced without clear major metabolites, which seemed to be one of the reasons that the metabolite prediction by the METABOLITE ID software did not match well with the experimental data due to the high degree of the secondary metabolomics pathways.

The pHLM metabolizing rate of CUMYL PEGACLONE, in the other hand, was relatively faster, completing within 2 hours with the high concentrations of metabolites. The alkyl chain hydroxylation was the major metabolizing process. The metabolism prediction by the METABOLITE ID also correctly projected the alkyl chain hydroxylation.

In the case of easily metabolized SCs, the pHLM incubation followed by LC-ESI-Q/TOF assisted by the METABOLITE ID procedure seemed to be effective in the prediction of metabolites, leading to the identification of the metabolites of new SCs.

Conclusions
With the lack of critical knowledge about the metabolism of emerging synthetic cannabinoids combined with no acceptable metabolite references, the in vitro pHLM incubation followed by LC-ESI-Q/TOF high resolution mass spectrometry, assisted by metabolite-predicting software, could be used as an alternative approach for the rapid identification of the SCs metabolites, in particular, for the forensic laboratories with the task to document the novel synthetic cannabinoids consumption in a few weeks.
Abstract ID  88
Origin of Ethanol in Postmortem Blood: an attempt to Stable Carbon Isotopic Characteristics Values (δ13C) in Forensic Casework.
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Aim
Alcohol tops the list of psychoactive substances encountered in postmortem toxicology. Ethanol analysis and results interpretation have always been a perpetual problem in forensic toxicology. The possibility of ethanol being produced in the body after death, e.g. via microbial contamination and fermentation is a recurring issue in routine casework. Derivatives of ethanol, e.g. n-propanol, ethyl glucuronide (EtG), ethyl sulfate, and other derivatives, have been used as biomarkers for a long time period. Recently, Chinese lawyers tried to question these biomarkers based on some logical interpretation. The prosecutors have posed such question to us “Are there new methods to explain the origin of ethanol by itself”.

In previous studies, we developed a gas chromatography - isotope ratio mass spectrometry (GC-IRMS) method to analyze the stable carbon isotopic characteristics (δ13C values) of ethanol in aqueous matrix. A preliminary study has shown a significant difference between drinkers’ blood and alcohol-disinfectant contaminated blood.

In this study, blood samples from postmortem cases were analyzed by GC-IRMS. Meanwhile, ethanol, n-propanol and EtG in these blood samples were analyzed as a contrast. The aim of this study was to explore the feasibility of origin determination of ethanol in postmortem blood using the stable isotope technique.

Methods
Blood samples were collected from 24 postmortem cases. Among these cases, 13 were traffic accidents, 8 were drowning cases, 2 were sudden deaths, and 1 deceased died from blunt force injuries.

The blood samples were analyzed by headspace GC, post column dilution - LC-MS/MS detection, and GC-IRMS for determination of alcohol and n-propanol, EtG, and δ13C values, respectively.

The determination of δ13C values by GC-IRMS analysis was performed as follows: samples were precipitated by acetonitrile, and then centrifuged at 12000 rpm for 5 minutes; afterward, the supernatants were filtered through 0.22 μm nylon membranes. An HPINNOWAX column was used for chromatographic separation. The CO2 reference gas and wine ethanol certified reference (BCR660) were used for isotope value calculation.

Results
The blood alcohol concentrations (BAC) of all 24 samples ranged from 1.76 mg/mL to 2.54 mg/mL. N-Propanol was not detected in blood samples from traffic accidents (from No.1 to No.13), and was detected in the remaining cases.

All 13 blood samples from traffic accidents were positive for EtG. EtG was also detected in No. 17 (drowning), No. 22 (sudden death) and No. 24 (blunt force injuries). The δ13C values of the blood samples from traffic accidents (n=13) and drowning cases (n=8) ranged from -31 % to -25 % and from -32 % to -8 %, respectively. The δ13C values of blood samples from cases No. 22, No. 23 and No.24 were -25 %0, -18 %0 and -26 %0, respectively.

As a statistical result, there was no statistical relationship between BAC and detection of n-propanol or EtG by variance homogeneity test (sig=0.013<0.05 for n-propanol, and sig=0.007<0.05 for EtG). Similarly, by bivariate correlation analysis, there is no relationship between the BAC and δ13C values (P<0.05, R<0.3).

Discussion
A brief review of the cases was as follows: The deceased in traffic accidents (from No.1 to No.13) were all drinkers; the deceased in the remaining 11 cases (from No.14 to No.24) were all putrefied. Among them, the deceased in No. 17 died by drowning due to alcohol intoxication. The deceased in No. 24 was in a violence fight in a bar. A large number of empty beverage bottles were found at the scene of the case No. 22. The analysis results of n-propanol and EtG are basically consistent with the details of the cases.

The δ13C values of the blood samples from traffic accidents present a relatively centralized interval. Based on previous research results, this interval can basically represent the δ13C value range of alcohol alive consumption. 64 % of the δ13C values of those blood samples collected from putrid bodies are not in the same range as the alcohol consumer, significantly (above -24 %).

There are still 4 complicated cases which cannot consistent with the certain rule form by others. This suggests that the stable isotope value of a single element may be limited. In the following research, we will introduce the stable isotope characteristics of hydrogen and oxygen.

Case No 19 requires special attention (δ13C value = -26 %0). There is no evidence that he drank before he died. However, the δ13C value of case 19 show the traces of alcohol consumption. According to the survey, he sticks to strict vegetarian diet. According to the transfer principle of δ13C value, it is an influential factor worthy of further study.

Conclusions
Stable isotope analysis shows its potential in origin determination of ethanol in postmortem blood. However, the matching ratio is still not enough only by carbon analysis. Especially for putrid bodies who may have consumed alcohol in their lifetime, effective data and conclusions are not available yet. The stable isotope values of hydrogen and oxygen will be further studied.
Abstract ID 114
Methcathinone induces oxidative stress to mediate neurotoxicity through activating apoptosis and autophagy.

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Aim
Methcathinone (MCAT), a designer drug abused as new psychoactive substance, has been questioned on neurotoxicity by unbalanced oxidative stress. To verify the doubt, the response of apoptosis and autophagy was determined after sub-acute exposure to high dosage injection of MCAT on rats. Subsequent correlation among oxidative stress, apoptosis and autophagy were analyzed further to explain the mechanism involved.

Methods
To address these issues, the biochemical markers of oxidative stress including glutathione peroxidase (GPx), glutathione reductase (GR), Glutathione-S-transferase (GST), catalase (CAT) and superoxide dismutase (SOD) were analyzed to evaluate the relationship between indicators and MCAT injection dose (0, 0.125 mg/kg, 0.25 mg/kg and 0.50 mg/kg). Mitochondrial membrane potential (Δψ), annexin V-FITC/PI, TUNEL and Western blot analysis (nNOS, LC3 and caspase3) were conducted in control and MCAT groups. Then, by an automatic device Columbus Auto-Track System, we measured the body temperature and stereotyped motor activity during rats model setting by 1.0 mg/kg MCAT and/or N-Acetyl-L-cysteine (NAC) injection in 15-day through 4 groups, including Normal Saline (NS), MCAT, MCAT+NAC and NAC. The indicators of oxidative stress (SOD, GSH, GSH-PX, GR, CAT and MDA), TUNEL and Western blot analysis were performed to determine how the apoptotic and autophagy pathways acting.

Results
The activity of anti-oxidative enzymes were found significantly lower in the brains of rats along with the doses of MCAT increasing. The apoptotic and autophagic effect of MCAT was proved compared with the controls. The hyperthermia and stereotypic motor activity were monitored in MCAT group compared with other three groups. Additionally, the inhibition of NAC was detected according to MCAT-induced activities of the anti-oxidative enzymes; NAC also alleviated MCAT caused-apoptosis and autophagy through TUNEL staining and expression of Atg-7, Beclin-1 and ratio of LC3-II to LC3-I, which showed decreased neurotoxicity according to quasi-simultaneous treatment of MCAT and NAC.

Discussion
MCAT exerts its effects by acting as a substrate-type releaser at monoamine transporters and increasing extracellular DA and 5-HT. After 15-day repeated drug exposure, stereotypic motor activity was evaluated in repeated administration paradigms, with great sensitization of repetitive movements due to psychostimulant effects. The hyperthermia was triggered by in vivo thermoregulatory properties of MCAT, maybe targeting activity at dopamine transporters (DATs) and serotonin transporters (SERTs) as well.

The relationship between the reduced and oxidized states of GPx, GR, GST, CAT and SOD, was considered an index of cellular redox status and a biomarker of oxidative damage, because of the indicators acting as a redox buffer. Oxidative stress is defined as a cytological consequence caused by imbalance between the production of free radicals and the ability to scavenge them. Oxidative stress also exhaust these dose-dependent various antioxidants enzymes.

Oxidative-induced apoptosis, as the most detrimental pathway in neurocytes, may contribute to several neural disorders. Our study indicated that MCAT exposure induced apoptosis in the brain of experimental rat models; while whether both the intrinsic and extrinsic apoptotic pathways are involved need further investigation.

Autophagy can be upregulated in response to oxidative stress, hypoxia or nutrient depletion. Previous studies demonstrated that antioxidants were able to attenuate generation of reactive oxygen and nitrogen species as well as partially inhibit autophagy and apoptosis induced by methylene and MDPV in SHSY5Y cells, supporting the role of autophagy as a cellular self-defense response against oxidative stress. That is why after exposure to MCAT, it was detected that autophagic characteristics were upregulated to cope with MCAT induced oxidative stress.

Conclusions
Conclusively, our main findings can be summarized as follows: 1) MCAT-induced stereotypic motor activity in rats could be partly inhibited by NAC. 2) oxidative stress associated related with concentrations was induced in the MCAT exposure rats, which could be blocked by antioxidant NAC. 3) apoptotic pathway appeared to be more activated in the MCAT exposure rats, which could be alleviated by antioxidant. 4) autophagy was also detected in the MCAT exposure rats and oxidative stress inhibitory could relieve relateddamage. Our results suggest MCAT induces neurotoxicity based on oxidative stress through activating apoptosis and autophagy.
Abstract ID 119
Evaluation of a New Application of Biochip Array Technology to the Simultaneous Screening of Drugs in Hair Samples on the Evidence Investigator Analyser.

V Anderson

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Aim
Testing for drugs of abuse in hair involves a non-invasive collection and provides a large window of detection as well as the history of drug exposure over time for an individual. Society of Hair Testing (SoHT) and European Workplace Drug Testing Society (EWDTS) guidelines for drug testing in hair are in place. To facilitate and increase the capacity of the screening step in the drug testing process, multi-drug detection offers advantages over single drug analysis. Biochip array technology (BAT) enables the determination of multiple analytes from a single sample. This study reports a new application of this technology to the simultaneous screening of amphetamine, benzodiazepines, benzoylecgonine/cocaine, cannabinoids, hydrocodone, ketamine, methamphetamine, opiates, oxymorphone and phencyclidine from a single hair sample.

Methods
The extraction of the drugs from hair samples (50 mg) involved washing with water and dichloromethane. The drugs were then extracted from the hair by centrifugation and addition of methanol and zircon pulverising beads. Following spinning and pulverisation, methanol was added, after decantation and drying the reconstituted sample was added to the biochip. For the screening of the extracted drugs, simultaneous competitive chemiluminescent immunoassays, defining discrete test sites on the biochip surface, were employed. The immunoassays were applied to the biochip analyser Evidence Investigator. With this system 54 biochips can be handled at a time. The total assay time including extraction was 100 minutes. Cut-off concentrations, inter-assay precision expressed as CV% (n=15 at -50% cut-off, cut-off and +50% cut-off) were determined. Cut-offs were selected to be as sensitive as possible whilst higher than the assay limit of detection. Authentic hair samples (n=43) were assessed with the biochip based technology and LC-MS/MS, the percentage agreement was reported.

Results
The assays presented the following cut-offs: 0.04 ng/mg (amphetamine), 0.02 ng/mg (benzodiazepines), 0.2 ng/mg (benzoylecgonine/cocaine), 0.01 ng/mg (∆9-THC), 0.001 ng/mg (THC-COOH), 0.04 ng/mg (hydrocodone), 0.5 ng/mg (ketamine), 0.05 ng/mg (methamphetamine), 0.02 ng/mg (opiates), 0.04 ng/mg (oxymorphone) and 0.02 ng/mg (phencyclidine). Cut-offs met or were lower than those recommended by SoHT and EWDTS. The inter-assay precision was <18% for all the assays at the concentrations tested. The assessment of authentic hair samples with the biochip based immunoassays and LC-MS/MS showed percentage agreement as follows: 98% (amphetamine, benzoylecgonine/cocaine), 95% (cannabinoids, opiates) 86% (methamphetamine).

Discussion
BAT allowed multi-drug detection from a single hair sample with lower cut-offs than SoHT or EWDTS guidelines for amphetamine, benzodiazepines, benzoylecgonine/cocaine, ∆9-THC, methamphetamine and opiates, reflecting high sensitivity. Authentic hair samples assessment using this methodology, showed favourable agreement with LC-MS/MS.

Conclusions
This biochip based application represents a useful multi-drug screening tool as it allows not only the detection of multiple drugs from a single hair sample but with a single platform (Evidence Investigator), multiple samples can be assessed at a time (up to 54 biochips can be handled at the same time).
Abstract ID 122

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Aim
Forensic laboratories have traditionally performed targeted drug screenings using GC–MS and/or LC–MS approaches, which use information obtained from analytical standards. However, using these targeted screenings, it is difficult to detect drugs, such as new psychoactive substances (NPS), for which we do not have sufficient information regarding retention time and fragmentation. Therefore, we aimed to build a non-target drug screening system using LC–QTOF–MS with in silico approaches. The system needs only chemical structures to predict retention times and ion fragmentation. To develop the data analysis procedure and conditions for LC–QTOF–MS, we examined whether it was possible to identify the following seven drugs (25B-NBPMe, 25C-NBOMe, 3-MeO-PCP, pentylone, α-PHP, PV8 and α-PVP) in the blood.

Methods
Drug analyses were performed using a Nexera X2 UHPLC system coupled with a LCMS–9030 QTOF equipped with an ESI ion source (Shimadzu). Chromatographic separation was achieved on a Kinetex XB-C18 column (100 × 2.1 mm, 2.6 µm) maintained at 40°C. The mobile phase comprised 10mM ammonium formate with 0.1% formic acid in water (A) and in methanol (B), and the flow rate was 0.3 mL/min. The gradient program was as follows: 5%–95% B between 0 and 7.5 min, 95% B until 10 min and 5% B from 10.01 to 15 min.
Non-target-screening analysis was performed using a data-dependent acquisition mode, for which mass range and collision energy were set to m/z 100–1000 and 10–60 V, respectively.

Ten nanograms each of the seven drugs were added to a 0.5 mL blood sample. The drugs were extracted using the QuEChERS method. The extract was injected into the LC–QTOF–MS.

Data analysis was performed using the ACD/MS Structure ID Suite software (Advanced Chemistry Development, Inc, ACD/Labs). For non-target screening, a drug database, including monoisotopic masses, predicted or experimental retention times and chemical structures, was created. Predicted retention times were obtained using the ACD/ChromGenius software (ACD/Labs). This program uses several calculated physicochemical properties to generate a prediction equation that is unique for every query structure. Drugs matching the retention time and accurate mass for precursor ions in the scan chromatogram were identified in the drug database. Search results were then ranked based on the mass fragments appearing in the active spectrum using the prediction function for mass spectrometry fragmentation.

Results
The accuracy of the prediction of retention time was examined using compounds for which retention times were measured. The predicted retention times of approximately 90% of the compounds were within ±1 min of the measured values, and approximately 65% of the compounds were within ±0.5 min. Therefore, we set the retention time windows of the database to ±1 min for the predicted values and ±0.2 min for the measured values to search drugs.

The ion chromatogram of the blood extract, including the seven drugs, generated over 10000 peaks. The developed drug search system reduced the candidate drug peaks to less than 20, and the seven drugs were included in the remaining peaks. This finding indicates that almost all the noise peaks derived from the blood and LC lines were successfully removed. Fragment analysis based on fragment prediction was then performed on the remaining peaks, which made it possible to reduce the number of candidate drugs.

Discussion
In non-target drug screening, it is important to exclude noise peaks from a chromatogram, especially in blood samples, wherein noise peaks are generated. Monoisotopic mass is the most effective parameter to identify drugs. However, using this technique, isomers cannot be distinguished. Therefore, it is also effective to use retention time for the identification of drugs. In addition, various filters, such as a cut-off value for peak height, were found to be helpful reducing noise peaks. Because the database, we created, includes monoisotopic masses and information about predicted and experimental retention times, we could reduce the number of candidate drugs considerably using a single search. Moreover, new drugs, such as NPS, can be easily added to the database with information about their chemical structures because the other information (monoisotopic mass and retention time) can be calculated from their structures.

Conclusions
We developed a non-target drug screening system for blood using LC–QTOF–MS with in silico approaches. The prediction of retention time and fragment ions was useful for identifying unknown drugs from the peaks of a chromatogram.
Abstract ID 125
Be careful when working in MRM mode: potential false positive results for 2-aminoindane.

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Aim
A patient came for the third time at the emergency department for ear-nose-throat symptoms, and finally died of a virulent group A Streptococcus pyogenes. As chemsex was evocated by the family, a screening for new psychoactive substances was undertaken.

Methods
General unknown screening was performed on 1 mL of serum. After basification, sample was submitted to a simple liquid liquid extraction before injection on an ultra high performance liquid chromatograph coupled to a TT 4600® high resolution mass spectrometer (UPLC-TOF-MS) from Sciex. We used a Kinetex C18 column with a mix of 5mM ammonium formate, methanol and acetonitrile as mobile phase. The mass acquisition method was a TOF survey scan from 50 to 1100 Da (cycle time 1 sec, accumulation time 0.150 sec), combined with a second experiment of product ion scan with an information dependent acquisition (IDA) method on a maximum of 20 candidates per cycle. The TOF is characterized by a resolution of 25000 at mass 829. Data were processed with the PeakView software 2.2, including a comparison to a library purchased by Sciex and regularly updated in-house.

Another method dedicated to 2-aminoindane was also developed on an UPLC-MS-MS (Quattro Premier®, Waters), using a BEH C18 column and a mix of 0.1% formic acid in methanol and 0.1% formic acid in water as mobile phase. The transitions monitored were 133.9 > 116.9 and 133.9 > 115.1.

Results
Analysis of the sample by UPLC-TOF-MS led to a match with 2-aminoindane, as indicated by the exact mass (134.1) and the 3 major fragments (91.0536; 115.0535; 117.0694). However, retention time of this compound wasn’t known, and slight differences were observed in the mass spectrum (e.g.: precursor ion present in the sample but absent in the commercial library).

2-aminoindane reference standard was then ordered to take a decision based on the retention time. Upon arrival (2.5 months later), reference standard was injected on UPLC-TOF-MS and its retention time was compared with the sample. A small difference of 0.3 minutes between the neat standard and the extracted serum was observed.

Then, we decided to spike the patient serum with 2-aminoindane, and two distinct peaks were observed. Finally, a retention time shift (0.1 min) was also observed in the LC-MS-MS method when analyzing the standard together with the patient sample.

Discussion
Knowing the retention time is useful for compound identification, and reference standards are required to be injected in the method. In our case, delivery time was so long (2.5 months) that it gave rise to a stability issue if we had to quantify the compound in the patient sample.

A small difference of 0.3 minutes between the 2-aminoindane neat standard and the extracted serum didn’t allow us to solve the problem, as some matrix effects can generate a small shift in the retention time.

In our case, as several mL of sample were luckily available (which isn’t a rule), we decided to spike the serum with 2-aminoindane, and two distinct peaks were observed, closing the case. However, an even smaller retention time shift (0.1 min) was observed in the LC-MS-MS method, as it was developed to be as fast as possible.

Based solely on the LC-MS-MS method, a false positive result would probably be unsuspected, unless ions ratios were calculated as a significant difference was observed for the sample compared to the neat standard (2.37 vs 6.62, respectively). Finally, about 900 other samples were tested with the methods, and the same interference was observed in 6 of them. Unfortunately, neither patient files analysis nor compounds found in the samples allowed us to elucidate the nature of this interfering compound.

Conclusions
Even if MRM mode is often preferred in order to increase the sensitivity, identification criteria - including retention time and two transitions - are sometimes insufficient to be 100% sure of the compound identification, especially if the runtime is short. Calculating ion ratios is indispensable and not optional if identification is to be based on MRM data.
Abstract ID 128

2C-B draws attention on the Belgian market: description of an amphetamine derivative.

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Aim
Two distinct and unrelated seized materials containing pills were submitted to the laboratory for the identification of the products. The first pill was a small yellow duck while the other one represented a green Super Mario’s face.

Methods
First, some classical identification methods including color tests were carried out for a rapid identification of sugars (Molisch reaction), starch (iodine test), cocaine (cobalt thiocyanate) and amphetamine derivatives (Marquis test). The powders are then diluted in methanol before analysis by high performance liquid chromatography coupled with a diode array detector (HPLC-DAD) from Waters on one hand, and ultra high performance liquid chromatography combined with a time-of-flight mass spectrometer (UHPLC-TOF-MS) from Sciex on the other hand. The quantification was carried out by HPLC-DAD.

Results
Only the Marquis test resulted in a coloration change (to green), while the other presumptive tests were negative. The chromatogram obtained on HPLC-DAD was dominated by a significant peak characterized by a retention time of 10.8 min and an UV spectrum showing maxima at 225 and 295 nm. A prominent peak was also found in UHPLC-TOF-MS after 5.54 min runtime, characterized by an exact mass of 260.0285 Da (duck pill) and 260.0279 Da (Mario pill). The corresponding mass spectrum showed 3 specific fragments at 243.0012, 227.9776 and 212.9541 Da.

Library matching of the mass spectra identified 4-bromo-2,5-dimethoxyphenethylamine, also called 4-BDMPEA or 2C-B, and the quantification provided a concentration of 9.71% and 5.35% for the Super Mario pill and the duck pill, respectively. Some traces of 2C-H were also identified in the duck pill.

Discussion
Obtaining a green color with the Marquis reagent is unusual for the phenethylamine derivatives mostly found in Belgium: Marquis reagent used to turn orange when exposed to (met)amphetamine and turn black with MDMA.

On UHPLC-TOF-MS, the difference between the theoretical accurate mass (260.0281) and experimental values was considered acceptable because lower than 5ppm (-0.8 for the Mario pill and 1.7 ppm for the duck pill).

Once identified in UHPLC-TOF-MS, the compound was added to the HPLC-DAD library and the match was confirmed. 2C-B is an entactogen drug with psychedelic and hallucinogenic properties. This amphetamine derivative was synthesized in 1974 by Alexander Shulgin and has already appeared in the drug market in the mid-1980’s. However, it’s obviously still popular on the drug market, even if it’s scheduled in Belgium.

Finally, traces of 2C-H were found in the “Duck” pill, which is a synthesis intermediate of 2C-B corresponding to the debrominated analogue.

Conclusions
Two pills containing 2C-B were identified for the first time in our Belgian laboratory. Even if this compound was discovered several decades ago, it is not forgotten by the party population, and thus requires proper identification capacities by the forensic labs.
Abstract ID 139
Rapid Screening of Alkyl Nitrites by Direct Analysis in Real-Time Time-of-Flight Mass Spectrometry (DART-TOF-MS) with Head-Space Injection
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Aim
Highly volatile alkyl nitrites have been regulated as the designated drugs, even before new psychoactive substances (NPS) became a prominent social issue in Japan. However, alkyl nitrites are still smuggled into Japan and are abused globally. Gas chromatography mass spectrometry (GC-MS) is commonly used to analyze alkyl nitrites, and head-space injection and solid-phase micro extraction are often employed with GC-MS because of the volatility of these compounds. However, GC-MS analysis is time-consuming because of the chromatographic separation process. The analysis time for alkyl nitrites may be substantially decreased by using direct analysis in real-time time-of-flight mass spectrometry (DART-TOF-MS), which allows direct analysis of any sample in the atmosphere. Our aim was to rapidly screen six alkyl nitrites regulated as the designated drugs in Japan with DART-TOF-MS and to identify their structural isomers.

Methods
DART-TOF-MS evaluation was performed by injecting the air phase into the ionization region of the DART ion source. Iso-propyl nitrite [C3H7NO2+H]+ (m/z 90.0462), n-butyl nitrite, iso-butyl nitrite, tert-butyl nitrite [C4H9NO2+H]+ (m/z 104.0709), iso-pentyl nitrite [C5H11NO2+H]+ (m/z 118.0863), and cyclohexyl nitrite [C6H11NO2+H]+ (m/z 130.0850), all regulated compounds in Japan, were measured by DART-TOF-MS at an ionization temperature of 300°C and orifice 1 voltage of 30 V. Sample injection of an alkyl nitrite for DART-TOF-MS was performed using the head space method as follows. Alkyl nitrite (0.1 mL) was injected into a glass vial that was sealed with a silicon septum cap, and then, the glass vial was heated in a dry block bath at 60°C for 10 minutes. The air phase in the glass vial was collected using a gas-tight syringe and injected for DART-TOF-MS.

Results
Detection of protonated alkyl nitrites was difficult because of fragmentation of the compounds. However, the mass spectra of the alkyl nitrites differed from one another owing to this fragmentation, and the characteristic fragment ions that formed after two hydrogens were desorbed from each protonated alkyl nitrite were detected at high intensity in iso-propyl nitrite, n-butyl nitrite, iso-pentyl nitrite, and cyclohexyl nitrite. The detected characteristic fragment ions were [C3H5NO2+H]+ (m/z 88.0393) in iso-propyl nitrite, [C4H7NO2+H]+ (m/z 102.0548) in n-butyl nitrite and iso-butyl nitrite, [C5H9NO2+H]+ (m/z 116.0719) in iso-pentyl nitrite and [C6H9NO2+H]+ (m/z 128.0710) cyclohexyl nitrite. In the tert-butyl nitrite mass spectra, the characteristic fragment ions that were not detected in other alkyl nitrites were observed. They were [CH2N2O2+H]+ (m/z 75.0188) and [CHN3O3+H]+ (m/z 104.0092). Furthermore, 2-butyl nitrite, which is the structural isomer of n-, iso-, tert-butyl nitrite, and n-pentyl nitrite, and 2-pentyl nitrite, 3-pentyl nitrite, neo-pentyl nitrite, sec-isopentyl nitrite, 2-methyl-1-butyl nitrite, and tert-pentyl nitrite, which are structural isomers of iso-pentyl nitrite, were measured by DART-TOF-MS. Therefore, in addition to identification of the six regulated alkyl nitrite compounds, characteristic ions due to fragmentation were detected with DART-TOF-MS measurement of these structural isomers. Furthermore, the thresholds of DART-TOF-MS measurement for alkyl nitrites were evaluated. The thresholds were set to the concentration in which the protonated alkyl nitrites were detected as the limit of detection (S/N = 3). As a result, the thresholds were as follows. Iso-propyl nitrite was 5% of iso-propanol solution; n-, iso, and tert-butyl nitrite were 1% of n-, iso, and tert-butanol solution, respectively; iso-pentyl nitrite was 5% of iso-pentanol solution; and cyclohexyl nitrite was 1% of cyclohexanol solution.

Discussion
Alkyl nitrites fragment during DART-TOF-MS measurement, making it difficult to detect protonated alkyl nitrites. However, the mass spectra following fragmentation differ, and characteristic fragment ions were identified for each alkyl nitrite. Therefore, it is possible to more rapidly identify regulated alkyl nitrites and their structural isomers by focusing on the characteristic ions generated by fragmentation than GC-MS. Furthermore, thresholds set for each alkyl nitrite can be useful in forensic tests, because the drugs most often seized contain high concentrations of pure alkyl nitrite.

Conclusions
This method enables rapid screening of alkyl nitrites for forensic science applications.
Abstract ID 144

The Cytotoxicity of the synthetic cannabinoids 5C-AKB48, 5F-MDMB-PINACA, ADB-CHMINACA, MDMB-CHMICA and NM-2201 on lung carcinoma and buccal carcinoma cell lines. Katharina Elisabeth Grafinger

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Aim

The biggest group of New Psychoactive Substances (NPS) are synthetic cannabinoids. These substances are designed to mimic the effects of marihuana. Both endocannabinoids (such as Δ9-tetrahydrocannabinol, the main psychoactive constituent of marihuana) and synthetic cannabinoids target the two cannabinoid receptors (CB1, CB2). However, Δ9-tetrahydrocannabinol is a partial CB agonist opposed to most synthetic cannabinoids, which are full agonists. Commonly, they are sold as herbal blends, for which synthetic cannabinoids have been sprayed on plant material, such as the shrub Turnera diffusa (damiana). The main form of consumption is smoking, and can lead to adverse effects or even death. The aim of the present study was to evaluate the cytotoxicity of the reference standard, herbal blend extracts and smoke condensates of five synthetic cannabinoids (5C-AKB48, 5F-MDMB-PINACA, ADB-CHMINACA, MDMB-CHMICA, NM-2201) on two different carcinoma cell lines. Most studies report only on the cytotoxicity of the reference standards of synthetic cannabinoids. However, because smoking is the main form of consumption, special interest was given on the cytotoxicity of smoke-condensates.

Methods

Reference standards and damiana were provided by the Institute of Forensic Medicine Freiburg. Herbal blend samples, obtained from the police were extracted with ethyl acetate and quantified with a five-point calibration using GC-MS. These results were used to prepare stock concentrations with the molarity of the herbal blend extracts and smoke condensates depending on the concentration of synthetic cannabinoid on the herbal blend material. An in-house smoke device was developed and optimised for the production of smoke condensates. These were qualitatively analysed using the same GC-MS method. In order to assess the cell viability the MTT assay was chosen, which mechanism is based on the metabolic properties of a cell to reduce the MTT when the mitochondrial functions are still intact. Because the main form of consumption of synthetic cannabinoids is smoking, the lung carcinoma cell line A549 (express CB receptors) and the buccal carcinoma cell line TR146 were chosen. Damiana extract and smoke condensates were simultaneously tested for cytotoxicity.

Results

The concentration of synthetic cannabinoid on the herbal blend samples ranged from 9.7 μg/mL to 19.7 μg/mL (5C-AKB48 19.7 μg/mL, 5F-MDMB-PINACA 9.7 μg/mL, ADB-CHMINACA 10.8 μg/mL, MDMB-CHMICA 17.3 μg/mL and NM-2201 19.0 μg/mL). The reference standards of SF-MDMB-PINACA, ADB-CHMINACA and MDMB-CHMICA lead to a significant concentration dependant decrease in cell viability, opposed for 5C-AKB48 and NM-2201, for which no significant changes in cell viability were seen (p<0.05 vs. vehicle or higher). Out of the five investigated reference standards, MDMB-CHMICA was the most potent one with an IC50 value of 17 μM in A549 and 19.3 μM in TR146. The herbal blend extracts of 5C-AKB48, SF-MDMB-PINACA, ADB-CHMINACA, and MDMB-CHMICA and of all smoke condensates lead to a concentration dependant decrease in cell viability (p<0.05 vs. vehicle or higher). For the reference standards of 5C-AKB48 and NM-2201 and the herbal blend extract of NM-2201 cell proliferation was observed.

Discussion

To the authors knowledge no studies have so far been reported on the cytotoxicity of reference standard, herbal blend extracts and smoke condensate, although the main route of consumption of synthetic cannabinoids is smoking. Results of four of the herbal blend extracts and five smoke condensates showed a significant decrease of cell viability. However, the influence of damiana has to be considered by comparison of the IC50 values. When the IC50 values are in the same range, the decrease of cell viability is solely from damiana itself. But, if the IC50 values of the synthetic cannabinoid are lower, the effects on the cell viability result from the synthetic cannabinoid. Opposed to this, higher IC50 values or no IC50 value mean that the synthetic cannabinoid is abrogating the effects of the damiana. The herbal blend extracts and smoke condensates of 5C-AKB48 and SF-MDMB-PINACA had IC50 values in the same range as damiana. Further, the herbal blend extracts of ADB-CMINACA and MDMB-CHMICA had lower IC50 values and are therefore more potent than damiana. Contrastingly, the NM-2201 herbal blend extract produced cell proliferation (no IC50 value could be determined), hence it was opposing the effects of the damiana leaves extracts.

Conclusions

The present study reports the comparative cytotoxicity of reference standards, herbal blend extracts as well as smoke condensates of synthetic cannabinoids. Out of the five tested compounds, MDMB-CHMICA was identified as the most potent substance. On the contrary, the NM-2201 reference standard and extract lead to an increase in cell viability compared to the control, suggesting that the herbal extract ablated the effects of the damiana leaves extracts. We would recommend to carry out further studies to assess potential pro-carcinogenic or tumorigenic effects of NM-2201.
Abstract ID 145
Investigating the ability of the microbial model Cunninghamhamella elegans for the metabolism of synthetic tryptamines
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Aim
Tryptamines can occur naturally such as in plants, mushrooms, microbes or amphibians, endogenous such as serotonin or as synthetic analogues. They are differentiatied into ergolines, with the most notorious being LSD or simple tryptamines. Tryptamines target different serotonin receptors, such as the 5-HT2A receptor, which is responsible for hallucinogenic effects. These substances have been on the recreational drug market and belong to the New Psychoactive Substances (NPS), due to their novelty. When it comes to NPS, metabolism studies are of crucial importance, due to the lack of pharmacological and toxicological data.

Different approaches can be taken to study in vitro and in vivo metabolism of xenobiotics. One such model is the zygomyctye fungus Cunninghamhamella elegans (C. elegans). It has been shown that C. elegans is capable to produce phase I and phase II biotransformation reactions and has the cytochrome P450 CYP 509A1 enzyme, which can facilitate reactions catalysed by the human CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4. The aim of the current study was to investigate the usefulness of C. elegans to produce tryptamine metabolites.

Methods
C. elegans was cultivated at 30 °C in liquid medium (total volume 30 mL each) in separate flasks for three days before the addition of 50 µM (800 µL prepared in 70% ethanol) of the four investigated naturally occurring and synthetic tryptamines [N,N-Dimethyltryptamine (DMT), 4-hydroxy-N-methyl-N-ethyltryptamine (4-HO-MET), N,N-di allyl-5-methoxy tryptamine (5-MeO-DALT) and 5-methoxy-N-methyl-N-isopropyltryptamine (5-MeO-MiPT)]. Experiments were carried out in duplicate and with two blank samples containing no drug solution. The reactions were stopped by freezing C. elegans at -20 °C until further analysis, which stops the metabolic activity of the fungi. Before analysis, the fungi biomass was homogenzized and both biomass and medium were extracted with liquid-liquid extraction using 1-chlorobutane. Metabolite identification was performed using liquid chromatography-high resolution-tandem mass spectrometry (LC-HR-MS/MS) with a quadrupole time-of-flight (QqTOF) instrument. Separation was performed on a Kinetex C8 column, 2.6 µM, 100 Å, 100 x 2.1 mm (Phenomenex, Basel, Switzerland), with a mobile phase consisting of water and acetonitrile, both with 0.1% formic acid. MS parameters were following: information-dependant data acquisition, positive mode, survey scan m/z 100-950, triggering the acquisition of product ion mass spectra for the nine most abundant precursor ions in a mass range from m/z 50-950. In silico predictions for data evaluation were performed using Meteor Nexus v.3.0.1 software (Lhasa Limited, Leed

Results
The data evaluation was performed using already published biotransformation pathways and the in silico predictions for all four studied tryptamines. Metabolites were identified according their precursor ion, fragmentation pattern and isotopic pattern. When available the metabolite mass spectra were compared to previously published data (4-HO-MET, 5-MeO-DALT and 5-MeO-MiPT).

C. elegans was capable to produce four out of the five DMT metabolites previously reported in human urine. However, it was not capable to rearrange DMT-N-oxide into the cyclization product 2MTHBC. For 4-HO-MET nine phase I metabolites were identified, of which six were previously reported by us in vitro (pHLM) and two in vivo (human urine). Two additional metabolites were found in C. elegans formed by oxidation of primary alcohols and carboxylation, respectively. C. elegans produced 16 5-MeO-DALT metabolites of which eleven have been previously reported in pHLM and rat urine. Specific C. elegans metabolites were formed by N-oxide formation and mono-,di- and tri-hydroxylation. The incubation with 5-MeO-MiPT in C. elegans resulted in six metabolites. Of those, we reported previously five in pHLM, four in human urine and three in human blood. One new metabolite, formed by demethylation, was identified.

Discussion
C. elegans was capable of producing all major biotransformation steps: hydroxylation, N-oxide formation, carboxylation, deamination, and demethylation. Comparison with previously published data showed that on average 63% of phase I metabolites found in the literature could also be detected in C. elegans. The most congruent result was for DMT with four out of five previously reported metabolites also found in C. elegans. Additionally, metabolites specific for C. elegans were identified formed by oxidation of primary alcohols, carboxylation, N-oxide formation, mono-,di- and tri-hydroxylation and demethylation.

Conclusions
From the obtained results we conclude that C. elegans is a suitable complementary model to other in vitro or in vivo methods, such as pooled human liver microsomes, human hepatocytes or the mouse model, to study the metabolism of naturally occurring or synthetic tryptamines.
Abstract ID 149
Development of a novel assay for drugs of abuse based on Molecularly Imprinted Polymers as synthetic antibodies.

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Aim
The aim of the work presented is to utilise molecular imprinting in the development of an easy to use assay for quantification of molecules of abuse. This project is primarily focused on detection of fentanyl, however the strategy employed for assay development can potentially be applied for many other analytes, as well as designer drugs, due to the generic nature of the assay format used. Considering the speed at which novel drugs are developed which circumvent standard analytical tests, the rapid fabrication of assays such as that described in this work offers a significant advantage compared to currently available techniques. The complete synthetic assay developed will have the potential to be a valid alternative to ELISAs and mass spectrometry technologies for use in drug identification, medical diagnostics, and rapid forensic detection.

Methods
Solid-phase nanoMIP synthesis are utilised to synthesise fluorescently-labelled molecularly imprinted polymer nanoparticles (nanoMIPs), using a chemically modified analogue of fentanyl as template for imprinting. The detection method used is an assay, molecularly imprinted nanoparticles assay (MINA), based on the same principle as a fluorescence immunoassay, where antibodies are substituted by nanoMIPs and the signal is generated by nanoMIPs themselves, due to the fluorescent moieties incorporated in the polymer. The assay is run in a standard 96-microtiter plate modified with magnetic inserts at the bottom of each well. Iron oxide nanoparticles (IO-NPs) are functionalised a template of interest are added to each well. When the nanoMIPs are added, if there is no binding between the nanoMIPs and the functionalised IO-NPs, nanoMIPs will remain in solution, and excitation light passing through the middle of the well will excite the fluorescent moieties incorporated in the nanoMIPs and a high signal will be recorded. If the nanoMIPs are binding the template immobilised on IO-NPs, nanoMIPs are driven down to the surface of the magnetic inserts, reducing the nanoMIPs in solution, therefore decreasing the signal intensity.

Results
NanoMIPs were successfully synthesised, size average 176 nm, and applied for the detection of fentanyl in two main assay formats. A displacement method was successfully developed. It requires an incubation step of nanoMIPs and fentanyl-functionalised-IO-NPs, whereas different concentrations of free fentanyl are successively added. A competitive method was also developed. In this case the three components are incubated at the same time and the limit of detection achieved was 21.94 nM (7.38 ng/mL). The developed assay is in the clinical relevant range of concentrations (0.5-100 ng/mL and comparable to most of the commercially available ELISA assays for fentanyl having a detection limit of 0.5 ng/mL, in addition MINA detection range is significantly larger than antibodies based assays, 7.4-598.2 ng/mL to 0.5-1.5 ng/mL.

Discussion
The assay format developed has the potential of becoming a commonly used technique for drug screenings in forensic and clinical laboratories. NanoMIPs are made by totally synthetic materials and for this reason no animal is required in the production chain. This avoids some of the already acknowledged problems such as antibodies poor reproducibility, due to differences in each animal immunosystem, in addition to the necessity of ethical approvals. This, plus the required storage at controlled temperatures (2-4°C) and the necessity of specialist personnel make immunoassays to be not ideally for in situ detection or clinical screenings, where highly specific facilities and trained staff is not always present. In these scenarios, screenings are mostly required within a limited period of time. The ease of use and short detection time of the developed novel MINA format are the main strengths of the developed technology.

Conclusions
Both displacement and competitive formats of MINA were developed to compare the advantages and disadvantages of each strategy and assess which would be preferential for the desired final application. The main advantage of using a MINA displacement assay is the potential to dry a nanoMIPs/template-functionalised IO-NPs solution on the surface of each well, with the ability to simply add a test sample and obtain a result with no further addition or separation steps necessary. The format is therefore very easy and straightforward for the end user, and due to this mix-and-read format allows results to be obtained very rapidly compared to a multistep ELISA assay.

Molecularly imprinted nanoparticle assay (MINA) was successfully developed to detect and quantify the concentration of fentanyl over the range 7.4-598.2 ng/mL, with a detection limit of 7.38 ng/mL.
Abstract ID 154

A novel method of determining tetrodotoxin (TTX) by UPLC-MS/MS applied in two death cases.

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Aim
Tetrodotoxin (TTX) is one of most potent low-molecular weight (~319 Da) neurotoxin found in some animal species, including pufferfish, newts and so on. The lethal dose that causes the death of 50% of a group of test animals (LD50) for TTX in mammals is respectively 2~10μg/kg intravenously and 10~14μg/kg subcutaneously. A novel method using UPLC-MS/MS has been developed in this study to determine trace concentration of TTX in the blood of two cadavers from two different kinds of death cases last year.

Methods
The samples of 0.5mL were precipitated with 1mL acetonitrile, then oscillated and centrifuged. After filtrating by hydrophobic membranes, the supernatant was analyzed by its retention time and two pairs of precursor/product ion pairs with ESI ionization mode and MRM detection mode in a UPLC tandem quadrupole mass spectrometry. The BEH Amide column (2.1*100mm, 1.7μm) and acetonitrile/water mobile phase system were used. The quality control samples (TTX spiked in blood) were operated in parallel. In order to evaluate the thermostability of TTX, a row of gradient concentration of TTX standard solutions were heated in water bath in 80 °C from 0 h to 3.5 h, which was a simulation of the pufferfish cooking. Samples were analyzed instantly after cooling down.

Results
The limit of detection (LOD) for TTX in blood was 0.1ng/mL with a good linear range of 0.5~200ng/mL. The intra- and inter-day relative standard deviations (RSDs) were below 4% and 5%. This method was applied to determine the concentration of TTX in blood instead of derivative method analyzed by GC-MS. In one case, the victim was assassinated through injecting the TTX, and the spectrogram containing a single peak was obtained as expected, which has been demonstrating the TTX concentration in 103ng/mL. In another case, the dead has eaten cooked pufferfish by mistake. A little but significant peak appeared in the spectrogram before the relatively huger main peak and it can not be ignored for qualitative analysis. However, the spectrogram of quality control sample has still shown the single peak clearly. In the spectrogram of the thermostability study experiment, the little peak appeared immediately when the sample has been heated and whatever its concentration was. The area of the little peak was growing with the heating time increasing and stable after 3h. The concentration in blood of the second case was determined finally in 101ng/mL by the main peak integration area.

Discussion
Thus, the difference of two cases in spectrogram could be related to the unstable thermostability of TTX. A further research to confirm the unclear structure was planned. In the very two cases, difference in spectrogram has reflected different ways of the same poison being into body, which could be a slightly potential hint for investigating in actual cases despite the inadequacy in the case quantity.

Conclusions
This study showed a rapid and credible method for analysis of TTX in blood which is suitable for TTX-involved murder and accident cases.
Presumptive color tests for design cathinones: Optimization of the test conditions for the novel Neocuproine reagent. Applications as a field test.

Luis Ferrari
Luis Ferrari
UM University

Aim
Synthetic cathinones (SC) or "bath salts" are a class of psychoactive substances that currently do not have an adequate and specific color test for presumptive identification. The color reagents as preliminary tests are very useful. Researchers proposed a method based on the reaction of cathinones with the Neocuproine-copper reagent, that was validated and compared with adulterants and mixtures of other NPS, however, they use high temperatures for more than 10 min. In our research, we have investigated catalysts to improve reaction times and other multicomponent tests (NPS and adulterants) such 2 and 3 Pyridine (Pyr).

Methods
Methyline, butylone, MDPV, alpha PVP and ethylone among others were investigated separately and with adulterants: caffeine, ephedrine, acetaminophen, levamisole (widely used as a cutting agent in South America), sucrose, lidocaine, benzocaine, protein powder, acesulfame, sucralose, codeine, and starch. Reactives: Neocuproine working solutions (0.01 M in 0.1 M hydrochloric acid (HCl), copper (II) nitrate 0.012 M in deionized water and sodium acetate 2.00 M. Using Pasteur pipettes adding a drop of reagents. Cu (II) (5 drops) and cathinone solution (5 drops) mixing gently. Then add: Neocuproine (5 drops) and water (3 drops) and added separately 2 drops 0.1 % 2 Pyr and after, the acetate buffer (5 drops) is added and then the color is observed. Once the color of the reaction is produced, photographs are taken under standardized conditions (angle of incidence of light, plate background).

Results
LOD for cathinone's tested: 15-20 µg. The colors were recorded by photography under standardized conditions, taking as a positive result of that intense orange-yellow. The substances tested as adulterants did not show interferences. Most of the amphetamines tested did not react with the reagent except methylenedioxy propiophenone, which showed a yellow-orange color but was fainter. Some precursors or synthesis reactants didn't give false positives (propiophenone and methylamine) the same for pyrovalerone structures, as MDPV.

Discussion
Law enforcement agencies and border protection agencies confiscate significant amounts of unknown and potentially illicit substances annually. The color test is an ideal presumption test and is commonly used to provide rapid, selective, economic and simple analyzes. Synthetic cathinones or bath salts are a class of psychoactive substances that currently do not have a proper and specific color test, used regularly for presumptive identification. This involves a prima facie evidentiary advance in the initial stages of a criminal investigation.

The Pyr produces the appearance of colors in times shorter than those published1 with high temperatures for more than 10 min. So, the most common cutting substances in our region do not yield positive results.

Since the original research using neocuproine and copper salts as color reagents use high temperatures for several minutes, our research was to test organic substances as possible reaction accelerators. This was used, 2 and 3 pyridines. As we will show, these substances accelerate the reaction without the need for heat at high temperatures. In addition, we verify that they do not interfere with the discrimination of cuts and adulterants.

Conclusions
We have tried the introduction of novel reactant Neocuproine-copper salt and organic substances that accelerate the reaction in order to give that manifests the yellow-orange color formed by cathinones and reactants, without the need to heat at high temperatures. Therefore it is suitable for field trials in confiscations of suspected substances (cathinones).

The present investigation about color test constitutes an advance for the application of the reagent in field trials.

Reference

(This is part of UM University Research N° PI3/18-02-AF-14. Criminalistic Department - Faculty of Law & Polit. Social Sci.).
**Abstract ID 157**

**Elucidation of elemental composition and structural properties of the nootropic drugs Picamilon and Aniracetam using LC-HRAM OrbitrapTM MS.**

Giampietro Frison

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Laboratory of Environmental Hygiene and Forensic Toxicology, DMPO Department, AULSS 3

**Aim**

Picamilon (4-nicotinoyl-aminobutyric acid) and Aniracetam (N-anisoyl-2-pyrrolidinone) are two nootropic drugs found in some dietary supplements.

The first drug was developed in the Soviet Union in 1969 and works by crossing the blood-brain barrier, after which it is hydrolyzed into gamma-aminobutyric acid (GABA) and nicotinic acid. The nootropic action is believed to be due to the CNS inhibitory properties of the released GABA, including anti-anxiety and anti-convulsant effects, as well as to the dilation properties of CNS blood vessels of nicotinic acid.

The second drug belongs to the racetam family and is presumed to act as a memory enhancer. Some evidence suggests that Aniracetam acts, through a glutamatergic mechanism of action, as a reversible positive allosteric modulator of AMPA receptors. In addition to its purported cognitive enhancement, it has also been investigated for its anxiolytic effects.

Several papers dealing with the determination of Picamilon and Aniracetam in both biological and non-biological samples have been published using various analytical techniques. However, to the best of our knowledge, no applications of liquid chromatography – high-resolution accurate-mass OrbitrapTM mass spectrometry (LC-HRAM OrbitrapTM MS) have been proposed so far.

Here we describe the analytical characterization, by means of LC-HRAM OrbitrapTM MS, of Picamilon and Aniracetam contained in two different seized powders sold as dietary supplements.

**Methods**

Methanolic/aqueous solutions (1 µg/mL) of the two powders were analyzed by means of LC-HRAM OrbitrapTM MS using a Thermo Scientific Accela 1250 UHPLC system equipped with a Hypersil Gold PFP analytical column (2.1 x 50 mm, 1.9 µm particle size), coupled to a Thermo Scientific single-stage Exactive HCD MS system, interfaced with an HESI-II source. Mobile phase A was water with 0.05% formic acid and 10mM ammonium formate, mobile phase B was acetonitrile with 0.05% formic acid. The flow rate was set to 400 µL/min. Mobile phase gradient was as follows: 99% A for 1 min, linear gradient to 70% B in 6.5 min, linear gradient to 100% B in 0.5 min, held for 2.0 min, column re-equilibration was performed with linear gradient to 99% A in 3.0 min, held for 3.0 min. MS was performed alternating full-scan (HCD off) and ‘all-ion fragmentation’ (HCD on, collision energy 25 eV) acquisition with a scan range from m/z 50 to 800 at a resolution of 100,000. No reference standards were analyzed.

**Results**

Accurate mass measurement of Picamilon and Aniracetam [M+H]+ quasi-molecular ions had a mass accuracy of 1.91 and 1.36 ppm, respectively. Furthermore, relatively abundant [M+Na]+ and [M+K]+ ionic species were obtained for both analytes. Moreover, fully superimposable experimental and calculated [M+H]+, [M+Na]+ and [M+K]+ isotopic patterns were obtained for the two nootropics, with relative isotopic abundance (RIA) values of 1.85 and 2.85 % for Picamilon and Aniracetam [M+H]+ ions, respectively. The isotopic fine structures (IFS) of the M+1, M+2, M+3 isotopic peaks (due to the contribution of 13C, 2H, 15N, 18O isotopes), relative to the monoisotopic [M+H]+ (M+0) peaks resulted completely in accordance with theoretical values for both substances.

Accurate mass measurement of the characteristic MH+ collision-induced product ions obtained from LC-HRAM OrbitrapTM MS analyses in MS/MS experiments were in full agreement with the expected structures and consistent with those previously reported using liquid chromatography-quadrupole mass spectrometry.

**Discussion**

The application of LC-HRAM OrbitrapTM MS allowed to obtain accurate mass measurement of Picamilon and Aniracetam [M+H]+, [M+Na]+ and [M+K]+ quasi-molecular ions in accordance with the elemental formulae C10H12N2O3 and C12H13NO3, respectively. The experimental and calculated [M+H]+, [M+Na]+ and [M+K]+ isotopic patterns, the RIA values and the IFS of the M+1, M+2, M+3 isotopic peaks confirmed the assignation of the above elemental formulae. Accurate mass measurement of the characteristic MH+ collision-induced product ions obtained from LC-HRAM OrbitrapTM MS analyses in MS/MS experiments enabled to obtain the structural characterization of both nootropic drugs.

**Conclusions**

The application of LC-HRAM OrbitrapTM MS may allow the elucidation of elemental composition and structural characterization of Picamilon and Aniracetam, hence improving the potential of their identification, as well as of other nootropic drugs, in both biological and non-biological samples, even in absence of reference standards.
**Abstract ID 159**

**LCMSMS vs GCMS in the detection of COC, EME, cinnamoylcocaine, hygrine and cuscohygrine in the real urine samples of coca leaves chewers after six years storage.**

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**Aim**
Chewing coca leaves or drinking coca tea is a legal practice in the Andean world (Bolivia, Perú, and Argentina), and this practice seems to be growing in urban populations in Argentina. The differentiation between people who chew coca leaf or drinking coca tea from people who consume cocaine (illicit abuse) is therefore very important in countries where these practices are legal. Both illicit cocaine and chewing coca leaves/drinking coca tea will give positive results when assessing cocaine and its metabolites in urine, oral fluid and hair. Recently, several legal disputes have happened to claim about cocaine positive results in urine are due to consumption coca leaves and not due to illegal use of cocaine. The forensic laboratory is obliged to keep biological samples for a certain period of time in order to reanalyze the samples if it is required by the attorney. The storage periods to which the samples can be subjected in forensic cases are variable may takes weeks, month and even years. Thus, the knowledge of the stability of the coca alkaloids is important in the Judicial process.

To evaluate the advantages or disadvantages of using LCMSMS or GCMS in the analysis of 13 urine samples from frequent chewers of coca leaves that were storage at -20°C, pH near 5 and NaF 1% for 6 years. As well as knowing the possibility to detect again the coca alkaloids present in the urine samples. Urine samples were analyzed in 2012 by GCMS and in 2018 by GCMS and LCMSMS. The alkaloids of coca leaves analyzed were cocaine (COC), methylecgonine (EME), cinnamoylcocaine (trCIN), hygrine (HYG) and cuscohygrine (CUS). The study has been focused on detecting HYG, CUS, and trCIN. HYG and CUS (and trCIN as a secondary marker) have been recently proposed as markers of chewing coca leaves and both compounds were found to be positive in the first analysis (year 2012).

**Methods**
Qualitative GCMS and LCMSMS methods were validated following international guidelines. Liquid-liquid extraction was the sample preparation technique used, and it required the use of MTBE and urine pH adjustment at 8.0. The validation parameters evaluated in GCMS and LCMSMS methods were: carryover, interference studies, and limit of detection; also, in LCMSMS the matrix effect (ionization suppression/enhancement) was evaluated. The LOD was established by GCMS (and LCMSMS) at 50 ng/mL (5ng/ml) for COC and trCIN, 100 ng/mL (10 ng/ml) for EME and 200 ng/mL (50ng/ml) for CUS. No interferences were found, no carryover to 500 ng/ml was observed by GCMS while carryover was present at 50ng/mL of COC and EME, at 200 ng/mL of trCIN in LCMSMS. No carryover was detected for CUS at the highest concentration of 500 ng/mL. The ME has not exceeded +/- 25%.

**Results**

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<tr>
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<tbody>
<tr>
<td>COC</td>
<td>84.6%</td>
<td>69.2%</td>
<td>100%</td>
</tr>
<tr>
<td>EME</td>
<td>100%</td>
<td>69.2%</td>
<td>92.3%</td>
</tr>
<tr>
<td>trCIN</td>
<td>61.5%</td>
<td>46.2%</td>
<td>92.3%</td>
</tr>
<tr>
<td>HYG</td>
<td>100%</td>
<td>0%</td>
<td>84.6%</td>
</tr>
<tr>
<td>CUS</td>
<td>100%</td>
<td>61.5%</td>
<td>84.6%</td>
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</table>

**Discussion**

HYG was detected 100% in urine samples by GCMS in the first analysis, while it was not detected in urine samples by GCMS in 2018; whereas, the number of urine samples positive to trCIN and CUS decreases 15% and near 40%, respectively. The LCMSMS performance is better than the GCMS technique when detecting COC and trCIN, and only two urine samples were negative for HYG and CUS (2018 analysis).

**Conclusions**
It is known that the conservation of urine samples with NaF 1%, acidic pH and temperature below -20C reduce the decomposition of ester-type drug like cocaine or methylecgonine which are readily hydrolyzed enzymatically and/or chemically (alkaline pH) (Logan B.K. 1994). However, it is scarce or there is no information about the stability of HYG, CUS, and CIN in biological samples. The disadvantage of this work is due to the use of a qualitative method that is unable to establish the extent that the compounds analyzed was transformed and how each type of stability contributed to the degradation of the analytes. Also, the long period (6 years) of storage of the urine samples do difficult maintain the same condition of the analysis prior to & after storage. In addition, the use of a more sensitive method with lower LOD as well as higher initial analyte concentrations in samples (urine samples are from frequent coca leaves chewers) allow analyte detection after long storage periods. LCMSMS should be taken into account as a technique in the case to reanalyze urine samples with a long storage period. Despite the satisfactory results in the analysis of the urine samples after a six years storage period, we should be cautious when using the data for Judicial cases about the legality or illegality of the origin of cocaine found in urine samples. There are not enough studies yet about the stability of trCIN, HYG, and CUS in urine samples.
Abstract ID 165
Application of liquid chromatography-high resolution mass spectrometry (LC-HRMS) to determine male sexual stimulant in selected food matrices.
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University of Technology Sydney

Aim
The wide availability and consumption of various food products claiming to enhance male sexual performance are often associated with intentional adulteration with phosphodiesterase 5 (PDE5) inhibitors and their analogues. Therefore, there is an urgent need to develop a sensitive and comprehensive analytical method to screen, identify, and quantify these adulterants in such complex matrices. In this study, the applicability of liquid chromatography-high resolution mass spectrometry (LC-HRMS) was explored to address this problem.

Methods
A total of 50 distinct brands of food products were acquired from Malaysia and Australia in forms of instant coffee premix, powdered beverage, honey, hard candy, jelly, and chewing gum. All samples were carefully selected based on their association with male sexual performance. Each sample was extracted using a modified QuEChERS extraction procedure. Then, LC-HRMS analysis was performed using an Agilent Technologies 1290 Infinity II liquid chromatography (LC) system coupled to an Agilent Technologies 6510 quadrupole time-of-flight mass spectrometry (QTOF-MS) instrument. Method development and validation were achieved based on 23 targeted PDE5 inhibitors and their analogues which were subsequently used for targeted identification and quantification. A personal compound database and library (PCDL) containing 95 known PDE5 inhibitors and their analogues was created using the PCDL software comprising a comprehensive collection of the compound names, molecular formulae and structures, and exact masses. This database was used to perform suspected-target screening of the adulterants in selected food matrices.

Results
The modified QuEChERS extraction procedure exhibited extraction recovery within 43.5% to 123.9% with insignificant matrix effects (ME) ranged from -9.2% to 8.8%. The applicability of the developed method was demonstrated with satisfactory validation results for each studied matrix. The screening approaches employed in this study (i.e. suspected-target and non-targeted) resulted in 41 positive samples. Out of this only 39 samples were identified and subsequently quantified against their respective certified reference materials. The procedure detected as much as five different analytes of PDE5 inhibitors in a single product. The amount of individual analyte and total analytes per dosage ranged from 0.05 mg to 148.39 mg and from 1.82 mg to 148.39 mg, respectively. In summary, the targeted analysis workflow successfully identified nine different target analytes and also detected another four highly suspected analytes. Based on the recommended dosage of approved PDE5 inhibitors, 17 samples were quantified exceeding the recommended limits per intended dose.

Discussion
The extraction procedure and analysis workflows proposed in this study proved to be excellent for determination of PDE5 inhibitors in selected food matrices. Insignificant ME allows accurate and precise quantification of PDE5 inhibitors which is essential for the assessment of risk associated with the consumption of adulterated food products, especially at high levels. Nevertheless, trace level quantification is equally important as some of these manufacturers may deliberately add various PDE5 inhibitors in trace amount into their products just enough to produce the claimed therapeutic effects but at the same time may avoid detection and quantification by relevant authorities.

Conclusions
Simultaneous screening, identification, and quantification of PDE5 inhibitors and their analogues in selected food products proposed in this study would be beneficial to tackle the problem of adulterated food products, especially with PDE5 inhibitors and their analogues to safeguard public health.
Abstract ID 167
Development of a non-targeted screening workflow for the detection of synthetic opioids in equine plasma.

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1University of Technology Sydney, 2Australian Racing Forensic Laboratory, 3Agilent Technologies

Aim
The continual evolution of the illicit drug market necessitates the development of non-targeted detection strategies. The production of certified reference materials (CRMs) often lags behind the introduction of new substances, therefore these strategies need to be able to function without relying on CRMs or library spectra. Synthetic opioids have recently emerged as a drug class of particular concern due to the health issues caused by their incredibly high potency. This research aims to develop a toolbox of complementary techniques which can be used for the non-targeted screening of synthetic opioids in biological samples. These techniques focus on data processing and data mining procedures allowing them to be utilised in conjunction with routine laboratory analysis methods.

Methods
Neat standards of 14 different fentanyl derivatives, 7 AH series opioids, 4 U series opioids, 4 W series opioids and MT-45 were analysed by liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) to evaluate collision-induced dissociation pathways. Fragments common to different subclasses of synthetic opioids were identified for use in product ion searching methods. Blank equine plasma samples were spiked with representative analytes from each subclass of opioids with concentrations ranging from 1 to 0.01 ng/mL. The spiked samples were extracted and analysed using routine methods at the Australian Racing Forensic Laboratory (ARFL). The data was then interrogated using a number of different non-targeted screening techniques including product ion searching, Kendrick Mass Defect (KMD) analysis and Agilent Technologies MassHunter Profinder (Recursive Feature Extraction) software. Following the detection of an unknown exogenous compound in a sample, compound identification techniques need to be developed to achieve putative identification.

Results
Following extensive evaluation of the dissociation pathways of different opioids, a number of common product ions were identified for different subclasses. Extracted ion chromatograms (EICs) were generated for these product ions, which indicated the presence of exogenous compounds at a concentration of 0.05 ng/mL. Additionally, KMD analysis was applied using a custom-built program developed in the Visual Basic for Applications (VBA) environment in Microsoft Excel. This program works off an m/z list generated from an averaged mass spectrum, making it vendor-software agnostic. Using a normalization factor of CH2 to calculate the Kendrick masses, the program successfully detected the presence of exogenous compounds at a concentration of 0.1 ng/mL with a reliability of ≥95% (n=21). Finally, the use of recursive feature extraction by Agilent MassHunter Profinder software has shown promise for the detection of opioids, with 8 of the 9 compounds present in the spiked samples being detected down to a concentration of 0.05 ng/mL.

Discussion
The techniques developed focus on the data processing and/or data mining aspects of analysis, making it easier for them to be used alongside routine analysis. The samples were all analysed using the routine methods applied at ARFL, meaning that there was no disruption to the day-to-day operation of the laboratory. For compound identification, evaluation of the dissociation pathways and the use of MSC allows for the exploitation of the high-resolution mass spectra generated during analysis. The effective application of MSC, however, relies on some basic understanding of the class of compound expected. Without some prior intelligence about the expected compounds, the evaluation of which possible molecular formula is relevant becomes increasingly difficult. Other compound identification techniques, such as artificial neural networks (ANNS), can be used for retention time prediction, allowing the analyst to take advantage of the often under-utilised retention time information to increase the confidence in the putative identification.

Conclusions
The non-targeted detection of drugs of abuse is becoming increasingly important in modern toxicology. A number of techniques have shown promise for the screening of synthetic opioids in equine plasma. These techniques can form part of a toolbox, allowing toxicologists to be better equipped to detect the presence of these compounds. Once developed, these techniques can be adapted to suit other biological matrices (such as human plasma).
Abstract ID 173
Assessment of concentrations of four phenothiazine antipsychotics in serum and whole blood using different diatomaceous earth–based solid-phase columns.
Takeshi Saito
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1Tokai University School of Medicine, 2Hiroshima University

Aim
We have been reported comparative results of phenothiazine antipsychotics concentrations in serum with whole blood concentrations extracted using various diatomaceous earth-based solid-phase columns (ChemElut, EXTrelut NT-1, ISOLUTE, and K-solute) in 2018 TIAFT. We reported that phenothiazine antipsychotics in whole blood was not detected in the ethyl acetate–extracted samples using the K-solute column. Therefore, in the present study we attempted to determine the concentration of phenothiazine antipsychotics in serum and whole blood samples using various diatomaceous earth-based solid-phase columns (ChemElut, EXTrelut NT-1, ISOLUTE, and K-solute) and elution solvents. We subsequently evaluated and compared the efficiency of these methods.

Methods
Whole blood and serum samples were collected from seven hospital staff volunteers who were not taking any medications. Whole blood and serum calibrator sample were prepared at 5, 10, 50, 100, 500, 1000, and 2000 ng/mL. For extracted samples, 5 ng of cyclizine (internal standard) was added to 20 μL sample. After adding 980 μL water, samples were subjected to columns. Dichloromethane-isopropanol (8:2), chloroform-methanol (9:1), diethyl ether, ethyl acetate, and methyl t-butyl ether were used elution solvents. Serum and whole blood samples were applied to each column. The sample was extracted with 5 mL elution solvent and the organic layer was evaporated to dryness. The residue was dissolved with 100 μL acetonitrile/0.1% acetic acid (80:20 vol/vol) and injected into the LC–MS/MS system. Chromatographic separation was performed on an InertSustain C18 HP 3 μm (3 mm × 100 mm) column at 40 °C using an Agilent 1200 LC system. The gradient conditions used for chromatographic separation were water with 0.1% acetic acid and acetonitrile. Detection on electrospray ionization (ESI) tandem mass spectrometry was achieved using an Agilent 6410 Triple Quadrupole Mass Spectrometer in the positive ionization mode. The ions were monitored using the multiple reaction monitoring (MRM) mode.

Results
All compounds were stable before sample preparation. The overall procedure was evaluated using authentic standard and differently spiked serum and whole blood samples (range, 5–2000 ng/mL phenothiazine antipsychotics). After extraction for all columns, IS was detected in serum and whole blood samples. Methyl t-butyl ether was effective for whole blood extraction using K-solute. The linearity of all compounds showed good results from 5 to 2000 ng/mL. Ethyl acetate and methyl t-butyl ether were effective for elution for both samples using EXTrelute NT1 and ISOLUTE. The linearity of all compounds achieved good results from 5–2000 ng/mL. Although ChemElut was effective using ethyl acetate for both samples, methyl t-butyl ether showed insufficient recovery. As a result, a large amount of solvent was needed for increasing recovery. Dichloromethane–isopropanol (8:2), chloroform–methanol (9:1), and diethyl ether were not able to detect all phenothiazines antipsychotics using all columns.

The recovery study was used at 3 concentrations (100 ng/mL, 500 ng/mL, and 1500 ng/mL). The total recovery rates of whole blood and serum were 18.5%–100.7% and 26.4%–98.8%, respectively. Although K-solute column was used methyl t-butyl ether, the others column was used ethyl acetate. Chlorpromazine sulfoxide, promethazine sulfoxide, and promethazine N-oxide were detected from whole blood and serum using all columns. Although oxidized compound peaks were quantitated using an authentic standard, they were equivalent to approximately from 3% to 10% of the unoxidized compounds.

Discussion
These results suggest that phenothiazines antipsychotics extraction from serum and whole blood using diatomaceous earth–based solid-phase columns seems to have an affinity with the elution solvent. However, the superiority of detecting these compounds varies according to the column used (including lot) and the matrix. Other peaks were not identified because the authentic oxidized standard was unavailable. However, the strongest influence on the observed peaks was attributable to the oxidized compounds of some position on the phenothiazine antipsychotics structures. Although whole blood and serum analysis is typically conducted in patients suspected of having been poisoned with promethazine, promethazine N-oxide, and sulfoxide compounds were detectable using ChemElut, EXTrelute NT1, ISOLUTE, and K-solute.

Conclusions
On the basis of this study, we conclude that ChemElut, EXTrelute NT1, and ISOLUTE are suitable extraction columns for evaluating phenothiazines antipsychotics in whole blood and serum.
Abstract ID 185
A cautionary tale of targeted LC-MS/MS: the case of an aripiprazole metabolite in urine and TFMPP.
Susan Grosse
Susan Grosse, Mark Tyler
Eurofins Forensic Services

Aim
LC-MS/MS targeted screening has replaced immunoassay screening techniques in many forensic laboratories, with one of the advantages being that LC-MS/MS is more specific than immunoassay, producing less false positives. The pitfalls of potential interferences with isobaric compounds or matrix interference have previously been reported and this work illustrates a further false positive result from a urinary metabolite of aripiprazole and further highlights the importance of questioning positive results that do not appear to fit with case circumstances.

Methods
In the laboratory, urine samples are screened by a rapid LC-MS/MS method which covers 80 common drugs of abuse and commonly prescribed medication. Identification of drugs is based on their Multiple Reaction Monitoring (MRM) transitions, retention time, and co-elution of stable isotopic analogues where available. Two aliquots of urine are simultaneously screened; one of the aliquots undergoes hydrolysis with the use of β-glucuronidase enzyme, the second aliquot is not hydrolysed. The method is fully validated and UKAS 17025 accredited; any issues from matrix interference and interference from other drugs were all addressed by conducting a thorough interference study with each compound and/or its common metabolite, including well-known isobaric compounds such as tramadol and o-desmethylvenlafaxine. Authentic urine samples were also analysed during the validation.

Results
In the beginning of 2018, it was noted that two urine samples from different cases produced a positive result for TFMPP (1-3-trifluoromethylphenyl)piperazine) on the hydrolysed urine sample; results that were not confirmed on the in-house LC-MS/MS confirmation method. This led us to believe a false positive for TFMPP was observed and an investigation into the root cause was conducted.

Discussion
The case circumstances were reviewed and it was noted that in both of the cases, the providers of the urine samples were prescribed aripiprazole. Aripiprazole, sold under the brand name Abilify in England and Wales, is an atypical antipsychotic, used in the treatment of schizophrenia and bipolar disorder. Investigations into the urinary metabolic pathway of aripiprazole showed that it metabolises to 2,3-dichlorophenylpiperazine (2,3-DCPP or DCPP), which is isobaric to TFMPP.

On review of the ESI spectrum for both TFMPP and DCPP, it would be possible to distinguish between the compounds based on the fragment ions, but the two product ions chosen for the TFMPP MRM in the current method (m/z 231.1 → m/z 288.0 and m/z 231.1 → m/z 118.1) are also present in DCPP. The reliability of data in forensic toxicology is of utmost importance. The possibility of false positives will have significant consequences if reported erroneously. Although the risk of false positives is lower in LC-MS/MS than immunoassay techniques, it is important that any results detected from a single method must be confirmed by a second technique, or tentatively reported within the context of the case. Thorough selectivity experiments during validation can prevent the misidentification of common drugs, although it is not possible to cover all potential drugs and metabolites in such studies. Therefore, even with more specific techniques, a separate confirmation method is required to minimise any false positives being reported.

Conclusions
The urinary metabolite of aripiprazole, DCPP, may produce a false positive for TFMPP, depending on the set up of your LC-MS/MS method. From our experience, it is generally only seen in urine samples that have undergone hydrolysis. Although the risk of false positives is lower in LC-MS/MS screening than immunoassay techniques, it is important that full validation is completed and that any results detected from a single method, must be confirmed by a second technique, or tentatively reported within the context of the case to avoid the potential for false positive results.
Abstract ID 190

In vitro phase I metabolism of the synthetic cannabinoid PX-1 by pooled Human Liver Microsomes and Cunninghamella elegans.

Patrick Dahm

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Aim

Synthetic Cannabinoids (SC) are substances that are highly metabolised. In urine samples, unmodified SC’s are rarely found and, therefore, urine screening relies on detection of SC metabolites. This requires knowledge on their metabolism. Metabolism data can be acquired via in vitro assays, such as human hepatocytes, pooled human liver microsomes (pHLM), cytochrome P450 isofoms and a fungal model, or in vivo by screening of authentic human samples or rat urine. pHLM is a commonly used assay for generating metabolites. The fungal model, Cunninghamella elegans LENDNER (C. elegans), is known to have a similar metabolism to that of humans, but is not widely applied in SC metabolism studies. SF-APP-PICA, also known as PX-1, shares structural similarity with AM-2201 (indole core and 5-fluoropentyl tail are identical) and AB-PINACA (carboxamide linker and 3-phenylpropanamide linked group are similar). However, there is a lack of published metabolism data regarding PX-1. Aim of this study was to examine the phase I metabolism in vitro metabolism of PX-1. For this purpose, two different in vitro models were used: pHLM and C. elegans.

Methods

PX-1 (0.101 mM) was incubated with pHLM for 3 hours at 37 °C, following a solid-phase-extraction. For fungal biotransformation, PX-1 (32.9 mM) was incubated with C. elegans for 6 days at 26° C. Following fungal incubation, the medium was extracted via liquid-liquid-extraction with dichloromethane. The organic layer was evaporated, reconstituted in ACN/H2O (v = 50/50) and purified by solid-phase-extraction. For both solid-phase-extractions EmporeTM Disk C18, 3M cartridges (Agilent Technologies, Santa Clara, CA, USA) were used. Samples were analysed in positive mode using an Agilent 6460 triple-quadrupole mass spectrometer equipped with a Kinetex® Polar C 18 column (100 x 2.1 mm, 2.6 µm; Phenomenex, Torrance, CA, USA), electrospray ionization (ESI) and coupled to a 1200 HPLC system from Agilent Technologies (Santa Clara, CA, USA). As mobile phase, 10 mM ammonium formate buffer with 0.1 % formic acid (A) and acetonitrile with 0.1 % formic acid (B) was used. Identification of phase I metabolites was based on mass and fragmentation data.

Results

From a total of 22 observed metabolites, pHLM and C. elegans produced 12 identical metabolites. Both assays showed a variety of monohydroxylations. The most abundant metabolite detected after pHLM and fungal incubation was produced by defluorination and monohydroxylation of the 5-flouropentyl side chain, which are typical metabolites for fluorinated and non-fluorinated SCs. The second most abundant metabolite observed with pHLM was formed by defluorination and amide hydrolysis. The two main metabolites of fungal metabolism are amide hydrolysis products, with or without defluorination. Incubation with pHLM showed, in contrast to fungal incubation, four different dihydroxylations, whereas fungus incubation showed only one dihydroxylated metabolite. Incubation with pHLM formed eight different monohydroxylated metabolites whereas fungal incubation only formed six of these monohydroxylated metabolites. Fungal incubation showed no dealkylated amide hydrolysed metabolite, which is formed by pHLM.

Discussion

Metabolites of PX-1 were characterized using pHLM and C. elegans in conjunction with LC-MS/MS. Amide hydrolysis was also observed in the metabolism of the structurally-related AB-PINACA, which has a similarly linked group; although a metabolic fluor-elimination is well known for fluorinated SC’s such as AM-2201. Fungal biotransformation showed higher levels of amide hydrolysis than pHLM.

Conclusions

Both the fungal, as well as the pHLM approaches, are in vitro methods which help to incorporate SC metabolites in screening methods in cases were no reference material is available. However, the relevance of the in vitro studies revealed metabolites in vivo that can only be assessed by investigation of urine from SC users.
Abstract ID 191

Chemical characterization of new psychoactive substances belonging to the class of synthetic cathinones in ‘legal high’ products.

Helena M. Teixeira

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Aim

Over the last decade, a high number of new psychoactive substances (NPS) were introduced on the drug market. The NPS consumption has grown in the last years when comparing to the rates of internationally controlled drugs, and the new trends in users and consumption patterns, as well as the new ways of communication, acquisition, and production of substances have challenged the conventional mechanisms of drug control. This new reality has led several European countries to interdict the NPS sailing, including Portugal. The island of Madeira was the first region of the country to promote legislation related with NPS prohibiting the sale and distribution of these substances due to dozens of hospitalizations between 2011 and 2012, with NPS being responsible for 2 deaths and around 190 hospitalizations up until October 2012. In 2013 a new Legislation was introduced in Portugal, prohibiting the advertising and marketing of new 159 psychoactive substances. Currently, synthetic cathinones are one of the NSP groups with the highest number of seizures recorded in our country, and since most of these substances are not detected in routine drug analysis, extra efforts must be made in order to develop new analytical strategies for the identification of these compounds.

The present study aims the chemical characterization of “legal high” products, suspected to contain synthetic cathinones by attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), gas chromatography coupled to mass spectrometry (GC-MS), and nuclear magnetic resonance (NMR). This study was developed under two doctoral programs funding by Fundação para a Ciência e a Tecnologia (FTC; SFRH/BD/116895/2016 and SFRH/BD/117426/2016).

Methods

Eleven seized products, “Flakka”, “Blast”, “Charlie”, “Kick”, “Bliss” (two packages) and “Bloom” (five packages), apparently containing synthetic cathinones, were provided by the Forensic Science Laboratory – Judiciary Police of Portugal. All samples were primarily analysed by ATR-FTIR, followed by GC-MS operated in electron-impact (EI) ionization mode. All mass spectra were compared with NIST 14 MS library and SWGDRUG MS library version 3.4. The characterization and structural elucidation were also realized by one- and two-dimensional NMR – proton nuclear magnetic resonance (1H NMR) and proton-proton correlation spectroscopy (1H/1H-COSY), respectively.

Results

ATR-FTIR spectra of products were consistent with the molecular structure of synthetic cathinones (intense peak at 1677–1699 cm⁻¹ (stretch C=O), intense peak at 1589-1603 cm⁻¹ (stretch C=C) and bands with relative low intensity at frequencies near to 3200 cm⁻¹ and 2426–2474 cm⁻¹ corresponding to an amine). Using GC-MS, it was possible to identify a total of seven cathinone derivatives, including methylone, methedrone, N-ethylcathinone, buphedrone, pentedrone, 4-fluoromethcathinone and α-pyrrolidinohexanophenone (α-PHP). Synthetic cathinones were found either as single component or in mixtures in the different products. N-ethylcathinone and buphedrone were the most commonly found synthetic cathinones in these products. Caffeine, a common active adulterant in illicit drugs, was found in several seized materials, including “Bloom”, “Bliss”, “Blast” and “Kick”, while ethylphenidate, a psychostimulant compound analogue of the prescription drug methylphenidate (Ritalin®), was detected in “Bloom” and “Charlie” products. Isopentedrone a by-product of the synthesis of pentedrone was also identified in one seized product (“Kick”). NMR analyses were also of great importance during the identification of synthetic cathinones. Methylone, one of the first ring-substituted synthetic cathinones to be detected in “Flakka” product. Isopentedrone a by-product of the synthesis of pentedrone was also identified in one seized product (“Kick”). NMR analyses were also of great importance during the identification of synthetic cathinones. Methylone, one of the first ring-substituted synthetic cathinones to be reported in the EU, was identified as the main component in “Bliss” products, while α-PHP was the main component detected in “Flakka” product.

Discussion

These results highlight the prevalence of synthetic cathinones in seized materials from the Portuguese market. Analytical standards are generally required for confirmation, but when standards are not available, mass spectrometry in combination with spectroscopic techniques are fundamental for the structural characterization of unknown compounds.

Conclusions

The identification of synthetic cathinones in seized materials was successfully achieved with ATR-FTIR, GC-MS and NMR analyses. ATR-FTIR was a particularly valuable tool, allowing a direct analysis with a small amount of recoverable sample. ATR-FTIR spectral bands proved to be useful for detecting functional groups characteristic of synthetic cathinones. On the other hand, the combination of GC-MS with NMR spectroscopy provides enough information for the unequivocal compound identification.
Abstract ID  192
Characterization of synthetic cannabinoids in ‘herbal incenses’ products.

Helena M. Teixeira
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Aim
The popularity of new psychoactive substances (NPS) among drug users has become a public health concern worldwide. Presented as “designer drugs”, NPS are synthetic compounds that mimic the rising effects of an original illegal drug, but with a slightly altered chemical structure, to circumvent legislation restrictions against illegal substances. The fast and unprecedented rate of evolution and spread of NPS, quickly gain expansion in the international market and, more importantly, the Portuguese market in the last few years. In 2011, this phenomenon spread in Madeira Island, and rapidly took over the continent like an epidemic.

Among NPS, synthetic cannabinoids (SCs) represent the largest, most diversified, and fastest growing group. The emergence and abuse of SCs has been increasing as an alternative to cannabis, since they mimic the effects of delta-9-tetrahydrocanabinol (THC), but with stronger and more potent effects. As their appearance on the drug market has been recent, and continue to emerge and attract new recreational users, it poses a steep challenge for clinical and forensic toxicology laboratories. The lack of comprehensively scientific studies on their toxicity and abuse liability, and the absence of appropriate analytical methods for their detection and identification contribute to this difficulty.

The aim of this work was the identification and characterization of herbal incenses suspected to contain SCs by gas chromatography coupled to mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR).

Methods
Nine seized products namely, “Caramba”, “Mauí”, “Mandala”, “Maya 2012”, “Esfinge”, “Atomic Bomb (strawberry)”, “Atomic Bomb (blueberry)”, “Radioactive (strawberry)”, “Radioactive (blueberry)”, suspected to contain SCs were provided by the Forensic Science Laboratory – Judiciary Police of Portugal. Each herbal incense was primarily extracted in methanol, and filtered before analysis by GC-MS in electron-impact (EI) ionization mode. All mass spectra were compared with NIST 14 MS library and SWGDRUGS MS library version 3.4. The characterisation and structural elucidation were realized by one- and two-dimensional NMR -proton nuclear magnetic resonance (1H NMR) and proton-proton correlation spectroscopy (1H/1H–COSY), respectively.

Results
By comparing the obtained mass spectrum to the reference mass spectrum of the NIST 14 MS library and SWGDRUGS library version 3.4, the literature and by analysing their fragmentation pattern, it was possible to identified 4 SCs of the naphthoylindole family, namely JWH-018, JWH-073, JWH-122 and JWH-210. As they belong to the same compound family, it should be expected that their structures and, their spectrometric behaviour, were similar. AKB-48 and XLR-11, two potent agonists for the cannabinoid receptors CB1 and CB2 were identified in “Maya 2012” herbal incense, as well as the presence of XLR-11cyclopropyl rearrangement product, due to its facile thermal degradation. Vitamin E, a natural metabolite usually found in the plant used as matrix in herbal incense products, was found in three herbal incenses ("Caramba", “Mauí” and "Mandala"). Oleamide, an eicosanoid, although in small concentrations was identified in almost all analysed products.

Discussion
NMR analysis was useful during the identification of SCs, being a complementary technique to GC-MS. Through 1H NMR and 1H/1H–COSY it was possible to identify JWH-018 and JWH-073 compounds, as the main components in 4 herbal products (“Atomic Bomb (strawberry)”, “Atomic Bomb (blueberry)”, “Radioactive (strawberry)”, “Radioactive (blueberry)”).

The analysis of 9 samples of herbal incenses has allowed the initial identification and characterization of 7 SCs (JWH-018, JWH-073, JWH-122, JWH-210, AKB48, XLR-11, MAM2201) by GC-MS and 1H NMR. Also, it was possible to identified tocopherol (vitamin E) and oleamide, two adulterants frequently added to herbal products in order to mask the active ingredients or added as preservatives.

Conclusions
The applied methodology proved to be usefull, allowing the preliminary identification of the different SCs in the mixture. Furthermore, the examination of mass spectral product ions, as well as the study of both one- and two-dimensional NMR experiments enabled the characterization of the molecular structure of SCs and may also assist the structure elucidation of these NPS.

This work was done within the scope of the protocol established between UMa and LPC/PJ with financial support by Fundação para a Ciência e Tecnologia (FCT) through two doctoral projects (SFRH/BD/116895/2016 and SFRH/BD/117426/2016).
Abstract ID 195
A Comparative study of Biochip Array Technology versus LC-MS/MS for the screening of NPS and DoA in 161 blood and urine from forensic and clinical casework.

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Aim
To evaluate the novel Biochip Array Technology (BAT) for the simultaneous detection of NPS and conventional DoA from a single run in 161 whole blood (B) and urines (U) samples from post-mortem and clinical casework.

Methods
80 B (46 deaths, 18 DUID, 6 suspected poisoning (SP), 3 DFC, 3 drug abuse DA, 2 homicides and 2 drug monitoring) and 81 U samples (25 workplace drug testing WPDT, 21 DA, 18 SP, 12 clinical investigations, 4 deaths and 1 drugs possession) were assayed on Evidence MultiSTAT (Randox®). 200 µL of a diluted B (1:4 sample diluent) or U were centrifuged, spiked into the biochip containing immobilized antibodies and run automatically. A competitive chemiluminescent signal is detected using a digital imaging camera and compared to that from a cut off material. The run lasts 17 min and allows the screening of: alpha-PVP, JWH18, UR-144, and AB PINACA derivatives, fentanyls, benzoylecgonine, amphetamine/MDA, methamphetamine/MDMA, THC, opiates, oxycodone, 6-MAM, methadone, buprenorphine, benzodiazepines, tricyclic antidepressants, barbiturates, tramadol, pregabalin, and EtG. The matching results (% agreement) of the BAT vs LC-MSMS, and the sensitivity/specificity (Sn/Sp, %) were calculated based on LC-MS/MS cut-offs. EtG assay was correlated to EtOH quantification by GC-FID (LOD= 60 ng/mL)

Results
BAT allowed the detection of newly marketed NPS like αPHP, MDPHP, pentylene, pentedrone and hexedrone in 4 U (agreement 86.2%). No blood sample was tested positive for alpha-PVP derivatives in both BAT and LC-MSMS experiments (n= 73). Interference with alpha-PVP assay in U was observed in the presence of tramadol (n=5) or venlafaxine (n=3) at concentrations > 2000 ng/mL. Three fentanyl (3-flurofentanyl < 1 ng/mL; ocfentanyl 284 ng/mL and fentanyl 3.3 and 4.2 ng/mL) and one synthetic benzodiazepines (etizolam: 125 ng/mL) were detected in U. Screening of synthetic cannabinoids was negative in all samples (n=143). Regarding conventional DoA, THC detected in both matrices with over 94% of agreement. THC-COOH≤ 7 ng/mL reported negative in 4 B in agreement with the cut-off (20 ng/mL). BZG was detected in 30 samples (match > 98%) except one urine reported 18 ng/mL BZG (cut-off 150 ng/mL). All opiates positive in U were detected by BAT (n= 62) and 6 B under the cut-off (80 ng/mL) reported negative (Sn/Sn: 81/100). 6-MAM was detected in 2 U (n=61), and oxycodone was screened positive in 4 cases (1B, 3U) with 4 False positive (FP) in B (Sn/Sn: 100/94) and 7 in U (Sn/Sn: 100/88) due probably to the presence of other opiates. Methamphetamine/MDMA screening reported concordant results in over 97% of samples. Two U with methamphetamine/MDMA < cut-off (200 ng/mL) reported negative. Amphetamine screening reported 84% of matching results (n=134) with 16 FP, 13 from a post-mortem B. The screening of other psychotropic drugs was concordant in over 87% of samples and EtG correlated better with EtOH in U (100% agreement) than in B (Sn: 59%, agreement 93%). For some failing test (n=5), a 1:8 dilution of B has been successfully applied to overcome high background noise.

Discussion
This novel screening method allows rapid detection of a wide panel of NPS and DoA, especially in post-mortem samples with a good agreement with LC-MS/MS results. The interference with alpha-PVP assay should be investigated as tramadol and venlafaxine present structural similarity and cross-react with the test. Amphetamine interference should also be addressed with regard to some putreafactive β-phenylethylamine bases, formed in post-mortem samples. Finally, the screening of mephedrone-like derivatives is recommended in future panel development as these molecules do not cross-react with pyrovalerone panel.

Conclusions
Abstract ID 196
Pharmacokinetics of Methylone and its Metabolites in Male Rats: Relationship to Brain Serotonin Depletion.

Marta Concheiro-Guisan

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Aim
Background: Methylone is a synthetic cathinone derivative that is the β-keto analog of ecstasy (MDMA). Although methylone and its analogs are increasingly popular drugs of abuse, limited studies have examined relationships between methylone pharmacokinetics, metabolism, and its pharmacodynamic effects.

Aims: The goal of the present study was two-fold: 1) to determine the pharmacokinetics of methylone and its three major metabolites- 4-hydroxy-3-methoxy-N-methylcathinone (HMMC), 3,4-dihydroxy-N-methylcathinone (HHMC) and 3,4-methylenedioxycathinone (MDC) in rat plasma and brain; 2) to relate pharmacokinetic parameters to pharmacodynamic effects including locomotor behavior, body temperature and post-mortem neurochemistry.

Methods
Methods: Male Sprague-Dawley rats (300-400 g) received s.c. injection of methylone (6, 12 or 24 mg/kg) or its saline vehicle (n=16/dose group). Groups of rats were decapitated at 40 min and 120 min post-injection, trunk blood was collected, and brains were removed. Blood was centrifuged to obtain plasma whereas brains were dissected to obtain prefrontal cortex, frontal cortex, and dorsal striatum. Plasma and brain tissue were stored at -80°C until analysis. Just prior to decapitation, rats were observed and rated for locomotor behavior using a numerical score, and core temperature was taken using a rectal probe. Plasma and prefrontal cortex were analyzed by liquid chromatography-mass spectrometry to determine concentrations of parent drug and its metabolites. Frontal cortex and striatum were analyzed by high pressure liquid chromatography-electrochemical detection to determine concentrations of dopamine, serotonin (5-HT) and their acid metabolites. All statistical analyses were performed with GraphPad Prism.

Results
Results & Discussion: Brain and plasma concentrations of methylone increased with increasing dose administered. However, methylone concentrations were greater than dose-proportional, indicating non-linear accumulation. The ratio of brain-to-plasma analyte concentration was determined at both time points and at the three different doses. In all cases, methylone and MDC showed a brain-to-plasma ratio >1, whereas HHMC and HMMC was <1. Cortical neurotransmitters were affected by drug treatment, such that methylone produced a dose-related decrease in tissue 5-HT. The methylone-induced depletion of 5-HT was detectable at 40 min but only reached significance at the 120 min time point (p<0.0001). At this latter time point, cortical 5-HT concentrations displayed a significant negative correlation with methylone concentrations in plasma (p< 0.0001) and brain (p<0.0005 ). Locomotor activity was significantly correlated with methylone at the 40 min time point only, but there was no correlation between body temperature and methylone.

Discussion
Conclusions: Methylone displays non-linear pharmacokinetics in plasma and brain, with methylone and its N-demethylated metabolite freely crossing the blood-brain barrier. We surmise that acute depletion of cortical 5-HT is related to methylone and MDC, but not its hydroxylated metabolites which do not effectively penetrate into brain.
Abstract ID 197

Determination of Methylone and its Three Major Metabolites in Rat Brain by Liquid Chromatography-Mass Spectrometry.

Nicole Centazzo

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John Jay College, City University of New York

Aim

Background: Synthetic cathinones are a relatively new set of drugs that present similar structural characteristics as amphetamines. Methylone, in particular, is the β-keto analog of 3,4-methylenedioxymethamphetamine (MDMA). Although fatalities due hyperthermia, serotonin syndrome, and multi-organ system failure in recreational users have been reported, limited data about its pharmacology are available. The development of specific and sensitive analytical methods in different biological matrices, such as plasma and brain, is critical to support pharmacokinetic and pharmacodynamic studies of this novel psychoactive substance. Analytical methods are available for the determination of methylone and their metabolites in plasma; however, methodologies in brain samples are scarce.

Aims: The main objective of this project was to develop an analytical method for the determination of methylone and its three main metabolites, 4-hydroxy-3-methoxy-N-methylcathinone (HMMC), 3,4-dihydroxy-N-methylcathinone (HHMC) and 3,4-methylenedioxyxycathinone (MDC) in rat brains by liquid chromatography tandem mass spectrometry (LC-MSMS).

Methods

Methods: Fifty mg of rat brain were weighed and suspended in 500 µL of 3% sodium metabisulfite (SMBS) 250 mM and 3% ethylenediaminetetraacetic acid (EDTA) 250 mM in 0.01M formic acid into 2 mL 1.4 mm ceramic beads homogenizer tubes. After homogenization in the bead mill and centrifugation, the supernatant was collected and 25 µL of the internal standard (methylone-d3 at 100 ng/mL) were added. The samples were incubated with β-glucuronidase from Red Abalone (Kura Biotec) to perform hydrolysis of possible phase II metabolism products. After cooling the samples to room temperature, 4-methylcatechol was added for chemical stability, and protein precipitation was performed with cold acetonitrile. The supernatant was collected and acidified before evaporation with a stream of nitrogen gas. The samples were reconstituted with 200 µL of mobile phase A (0.1% formic acid in water), centrifuged and filtered by nano/FV® PVDF 0.2 µm vial filters (Thomson Instrument Company). Twenty µL of the filtrate were injected into the LC-MSMS (LCMS-8050, Shimadzu) system with electrospray (ESI) in positive mode. The chromatographic separation was performed on a Synergi Polar reversed phase column 2.1x100mm, 2.5µm (Phenomenex), employing a gradient of 0.1% formic acid in water and in acetonitrile, with a total run time of 9 min. Two multiple reaction monitoring (MRM) transitions were monitored for methylone (207.8>160.2, 207.8>132.2), all three metabolites (195.8>160.1, 195.8>132.1 for HHMC, 209.9>160.1, 209.9>132.2 for HMMC, 193.8>146.1, 193.8>118.1 for MDC) and methylone-d3 (210.9>163.15, 209.9>135.25). The method was validated evaluating linearity (n=6), intra and inter-day imprecision (n=15), bias (n=15), extraction efficiency (n=6), matrix effect (n=9), and process efficiency (n=6). Imprecision, accuracy, and extraction efficiency were evaluated at two concentrations, low QC (15 ng/g) and high QC (750 ng/g). As a proof of concept, an authentic sample obtained 120 post-exposure from a rat dosed with 6 mg/kg methylone was analyzed.

Results

Results & Discussion: The method was linear from 5 to 1,000 ng/g with 1/x weighting, except methylone that used 1/x2 weighting. The intra-day imprecision was 4.3-6.3% at low QC and 8.6-12.8% at high QC. Inter-day imprecision was 2.5-3.7% for low QC and 3.5-5.3% for high QC. The bias at low and high QC ranged from -7.8 to 0.5%. Matrix effect for the target analytes ranged from -68.5 to 36.5% (CV<7.2%, n=9), while process efficiency and extraction efficiency were 13.1-124.5%, and 39.8-91.3%, respectively. The authentic sample was positive for methylone and its metabolites; the concentrations found were 288.5 ng/g for methylone, 493.4 ng/g for MDC, 17.1 ng/g for HMMC and 5.2 ng/g for HHMC.

Discussion

Conclusions: We developed an analytical method to determine methylone and its three metabolites, HMMC, HHMC and MDC, in rat brain samples with high sensitivity (LOQ 5 ng/g in 50 mg of sample) and specificity (retention time, 2 MRM transitions per analyte, ion ratio). The developed method will be applied to authentic rat brain samples dosed with different methylone amounts, allowing the study of the pharmacokinetics and pharmacodynamics of methylone and its metabolites in rats.
Abstract ID  216
Quantification of ethanol in whole blood by extraction using NeedlEx® and gas chromatography/mass spectrometry.

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Aim
Ethanol is a most popular beverage, but its excessive consumption causes poisonings, fatal accidents, and many violent crimes. Blood ethanol quantifications are mandatory in clinical and forensic toxicological investigations. NeedlEx®, a new extraction needle, is a device that concentrates volatile organic compounds (VOCs), such as alcohols, organic solvents, amines, and fatty acids. To the best of our knowledge, there are no reports of quantification of ethanol in whole blood by NeedlEx®-gas chromatography/mass spectrometry (GC/MS). Accordingly, in this study, we developed and validated a new quantification method for ethanol in whole blood. This method was successfully applied for the quantification of ethanol in whole blood samples collected from autopsy cases.

Methods
We used NeedlEx® for alcohols (Shinwa Chemical Industries, Japan), which adsorbs monohydric alcohols with up to 3 carbon atoms, and solvents, such as acetone and diethyl ether, with boiling points below 100°C. Whole blood samples (0.1 mL) and 0.1 mL IS solution (1 mg/mL ethanol-d6) were incubated at room temperature in a 10-mL headspace vial. The NeedlEx® for alcohols with a gas aspirating pump was exposed to the headspace of vial to allow adsorption of VOCs. The GC separation of the compounds was achieved on a fused-silica capillary column Rtx-5 ms (30 m x 0.25 mm i.d.; 0.25-μm film thickness) with MS detection operated in electron impact ionization ion source mode. The method for ethanol quantification was validated by characterizing a series of parameters, such as linearity, accuracy, and precision (intra- and interday) according to the US FDA guidelines on bioanalytical method validation.

Femoral vein and right heart blood samples were obtained from autopsy cadavers at Aichi Medical University in 2017.

Results
The peaks of ethanol and ethanol-d6 in selected ion chromatograms were clearly visible, with retention times of 1.0 min, and there were no peaks that interfered with the measurements. The calibration curve showed a good linear relationship in the range from 0.1 to 5.0 mg/mL, with a correlation coefficient of 0.998. The accuracies and precisions at concentrations of 0.1, 0.3, 2.0, and 4.0 mg/mL were 99.0–112% and 3.0–7.0%, respectively. Overall efficiencies including both extraction recovery and matrix effect were determined at the low (0.3 mg/mL) and high concentrations (4.0 mg/mL) by comparing whole blood samples with solutions of reference standard ethanol dissolved in water (n = 5). They were 108% for 0.3 mg/mL and 104% for 4.0 mg/mL, suggesting that the extraction recovery and matrix effect showed almost the same results as described in previous reports by headspace GC/MS.

We analyzed the 17 blood samples collected at autopsies. These samples were taken from femoral veins (n = 4) and right hearts (n = 13) and were previously checked to contain ethanol by GC-FID. Ethanol was detected in all samples, and the median concentrations were 0.72 mg/mL for femoral vein blood and 0.31 mg/mL for right heart blood.

Discussion
In this study, a new quantification method was developed for analysis of ethanol in whole blood samples by NeedlEx®-GC/MS. The calibration with a good linear relationship in the range from 0.1 to 5.0 mg/mL showed a high degree of linearity within this range. The accuracies (99.0–112%) and precisions (3.0–7.0%) were acceptable at all concentrations. The method was successfully applied to actual analyses of autopsy samples. These results indicated that ethanol could be quantified in whole blood samples collected at autopsies by NeedlEx®-GC/MS. Real advantage of this method is that ethanol can be extracted selectively by NeedlEx® and analyzed precisely by GC/MS. Thus we consider that this method also has its applicability to a number of other volatile compounds in clinical and forensic toxicology. One of the current disadvantages of this method is the limitation in the number of target compounds, such as 1-propanol, which can be produced during decomposition and putrefaction processes. Therefore, further studies are needed to establish a method that can simultaneously quantify ethanol, methanol, 1-propanol, and other VOCs in whole blood. In our preliminary experiments, the method can detect methanol at poisoning concentration about 200 μg/mL. Additionally, quantification of ethanol using the present method was possible at a lower level of 2.5 μg/mL. These results suggested that the present method may be applicable to clinical and forensic toxicology.

Conclusions
In this study, we developed and validated a method involving NeedlEx®-GC/MS for the quantification of ethanol in whole blood. The applicability of this method was demonstrated by measuring the concentrations of ethanol in whole blood samples from autopsies. This method was found to be suitable for forensic and clinical toxicology analyses.
Abstract ID  229
Determination of ocfentanil and W-18 in a suspicious heroin-like powder in Belgium.
Maarten Degreef
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Aim
Since the early 2010s, synthetic opioids have been on the rise in the illicit drug markets of Europe and North America, often as adulterants or substitutes for heroin. Ocfentanil, an early-onset fentanyl analogue, has been implemented in several fatalities, predominantly in Europe. W-18 is more prevalent in Canada and the United States. We present the findings following the analysis of an unknown brown powder, advertised and purchased as heroin, which was sent to our laboratory under the framework of the Belgian Early Warning System on Drugs.

Methods
The sample was screened by direct injection of 1.0 µL of the dissolved powder (diluted to 1 mg/mL in either methanol or ethyl acetate) on both a liquid chromatograph (LC) coupled to a diode array detector (DAD) and a gas chromatograph (GC) coupled to a single quadrupole mass spectrometer (MS). To confirm the results the sample was further diluted with methanol to 1 µg/mL, of which 0.5 µL was injected on an LC coupled to a triple quadrupole mass spectrometer (QQQ). The analytical method was designed for the detection and quantification of fentanyl, 31 of its analogues, W-15 and W-18, operating in dynamic multiple reaction monitoring mode. For ocfentanil, the monitored transitions were m/z 371.2 to 105.1 (100%) and 188.2 (90%), for W-18 m/z 422.1 to 111.1 (100%), 273.1 (45%) and 174.9 (25%).

Results
LC-DAD and GC-MS screening revealed the presence of the new psychoactive substances (NPS) ocfentanil and W-18. The acquired spectra were compared to those in our in-house database (standards for ocfentanil and W-18 were purchased from Chiron AS, NO). Results from the GC-MS screening were also compared to the Cayman Spectral Library (CSL). The LC-QQQ analysis was selective for fentanyl, 31 of its analogues, W-15 and W-18, and confirmed the findings. All monitored transitions had ion ratios within ± 20% of those determined during method optimisation. As no labelled internal standards were available, retention times were checked by subsequent injection of reference standards. Semi-quantitative LC-DAD analysis showed ocfentanil and W-18 made up 1.6% (m/m) and 0.3% (m/m) of the powder respectively. Common cutting agents paracetamol (56% m/m), caffeine (24% m/m), benzoic acid (9.5% m/m) and quinine (trace amounts) were also detected.

Discussion
Despite the powder being sold as heroin and its striking resemblance to it in consistency and colour, no heroin, 6-monoacetylmorphine or morphine were found. Our analyses revealed the presence of the fentanyl analogue ocfentanil and of W-18. Similar ocfentanil concentrations were reported previously in powders from Belgian origin. To our knowledge this is the first reported sighting of both compounds together in a powder, which has prompted an alert waring by the Belgian Early Warning System on Drugs.

A significant mismatch was found between the W-18 spectrum in our in-house library and that in the CSL, in part due to the absence of the molecular ion at m/z 421. This could be of particular importance as it may have resulted in an underreporting of cases.

Conclusions
The unknown powder tested positive for ocfentanil and W-18. To the authors’ knowledge this is the first case of the combined detection of these two new psychoactive substances in the same powder. We also demonstrate the advantage of building an in-house reference library beyond retention time.
**Abstract ID 242**

**Metabolomic analysis of different cannabis species by untargeted HPLC-HRMS.**

Christoph Hassenberg

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**Aim**

The cannabis plant is used for a variety of purposes. The aim of the work was to examine whether or not the cannabis species from fiber hemp, CBD hemp, various medicinal cannabis and illegal cannabis can be differentiated via their metabolome. Particular focus is given to the determination of markers for differentiating between medicinal and illegal cannabis species.

**Methods**

For the purposes of analysis, cannabis buds from five different fiber hemp species (abbr. MSH; varieties finola (n=3), felina (n=1), fedora (n=1)), 11 different CBD hemp species (abbr. CBH; Switzerland (n=10), Germany (n=1)), 11 different medicinal cannabis species (abbr. MED; Dutch products (n=5 x 2 lots, e. g. Bedrolite®), a Canadian product (n=1) - orderable in the period 09-12/2018) and 45 different illegal cannabis species (abbr. STR) were extracted in 70% methanol/water in an ultrasonic bath (in triplicate for MSH, CBH and MED; single termination for STR). After addition of the internal standard methaqualone, the extracts were measured using the Q Exactive Focus ™ Quadrupole-Orbitrap Mass Spectrometer (HRMS) in the full MS mode, followed by data dependent MS2 - fragmenting the three most abundant ions of each MS1 scan. A total of 126 chromatograms of 45 STR, 15 MSH, 33 MED and 33 CBH samples were analyzed with Compound Discoverer ™, after which statistical data analysis was carried out with MetaboAnalyst 4.0.

**Results**

The 3D-Principal Component Analysis (PCA) plot of the four groups demonstrates that, in the metabolome, the MSH and CBH are clearly distinguishable from the other cannabis species, whereas MED and STR could not be distinguished from each other. As expected, the low tetrahydrocannabinol (THC) and tetrahydrocannabinolic acid (THCA) content along with the distinctive content of cannabinoid (CBD) and cannabidiolic acid (CBDA) in MSH and CBH, are mainly responsible for their uniqueness. Sparse partial least squares discriminant analysis (sPLS-DA) of the MSH/CBH groups additionally revealed significantly higher levels of catechin, epicatechin, cannabigerol (CBG), cannabigerolic acid (CBGA) in CBH, and significantly lower levels of safrole, in comparison to that in MSH.

In the principle component 1 / principle component 2 (PC1 / PC2) plot, the medicinal cannabis product Bedrolite®, with low content of THC and high content of CBD, is located next to the CBH. This emphasizes the importance of THC, THCA, CBD and CBDA for differentiation. However, significantly higher levels of catechin and CBDV in Bedrolite® allowed the differentiation between CBH and CBD-rich/THC-poor MED. In order to examine the possibility of differentiation between MED and STR, a sPLS-DA of these two groups was done. With such a model, one can distinguish between MED and STR by taking the first three PCs into account. By leave one out cross validation (LOOCV), a 0 % error rate for correct classification was confirmed for the model. Some 38 potential markers were selected and an undirected PCA subsequently was carried out. The various MED products with high CBD/low THC content are clearly distinguishable one from the other; those with nearly equal CBD/THC-levels are distinguishable from illegal cannabis; whereas THC/-rich and low-CBD MED closely resemble STR. The importance of THC, THCA, CBD and CBDA as markers is, once again, underlined. Additional markers for differentiation of MED and STR are e.g. the cannabinoids CBG and CBGA, as well as the flavonoids catechin and epicatechin. Furthermore, nicotine, amphetamine and methamphetamine were identified in STR as external contaminants, presumably arising from handling and packaging of STR and other drugs.

**Discussion**

**Conclusions**

Overall, it could be shown that, by means of untargeted LC-HRMS and statistical analysis, the species of fiber hemp, CBD hemp, medicinal cannabis and illegal cannabis can, in principle, be differentiated one from the other, based on their metabolome (with the caveat that, THC-rich medicinal cannabis / illicit cannabis awaits validation by quantitative determination of markers and statistical testing in a future study). THC, THCA, CBD and CBDA were identified as important markers, along with other cannabinoids such as CBG and CBGA, as well as the flavonoids catechin and epicatechin. Furthermore, nicotine, amphetamine and methamphetamine were identified in STR as external contaminants, presumably arising from handling and packaging of STR and other drugs.
Abstract ID 243
Assessment of biased agonism amongst distinct synthetic cannabinoid receptor agonists scaffolds.
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Aim
Cannabinoid receptors are considered as key drug targets for a number of diseases. As of today, most of the typical orthosteric cannabinoid ligands still provoke adverse (psychotropic) side effects that impair their therapeutic utility. Although the research on biased signaling of synthetic cannabinoid receptor agonists (SCRAs) is still in its infancy, the outcome of these studies could contribute to the development of compounds that are signaling pathway-selective, rather than only receptor-selective, hence not evoking on-target adverse effects. This is the first study to report on the screening for ligand bias in an extended panel of SCRAs, chosen to cover a broad diversity in chemical structures (i.e. 2 SCRAs per class of chemical structures). Therefore, we have developed and applied bio-assays based on the recruitment of different transducers (Gαi-protein or β-arrestin2) to the activated cannabinoid receptor 1 (CB1) to assess the occurrence of biased signaling among SCRAs. This approach allows a better insight into the structural features of certain SCRAs that provoke biased signaling, which ultimately may aid the development of new therapeutic compounds with less unwanted psychoactive effects.

Methods
The bio-assays implemented in this study are complementation-based assays that require the fusion of both the transducer and the CB1 to one part of a split luminescent protein, the Nanoluciferase. The development of the CB1 activation bio-assay based on the recruitment of β-arrestin2 in a stable HEK293T cell system has been reported previously (Cannaert et al., Anal Chem, 2017). A similar approach, based on retroviral transduction, for the generation of a stable HEK293T cell system co-expressing the CB1 and the mini Gαi-protein was executed in this study. Both stable cell lines, based on the recruitment of the mini Gai-protein or β-arrestin2, were used for the simultaneous screening of a panel of 21 SCRAs, encompassing distinct structural classes, including: Cyclohexylphenols ((C8)-CP 47,497), Naphthoylindoles (AM-2201, JWH-018), Benzoylindoles (WIN48,098, RCS-4), Phenylacetylindoles (JWH-250, RCS-8), Alkoylindoles (XLR-11, UR-144), Ind(az)ole esters/carboxylates (SF-PB-22, BB-22), Ind(az)ole carboxamides (SF-APINACA), Amino acid derivatives (MDMB-CHMICA, MMB-CHMICA, (S)-SF-MDMB-PINACA, 4F-MDMB-BINACA), Cumylamine derivatives (SF-CUMYL-PINACA) and Carbazoles and γ-Carbolines (CUMYL-PEGACLONE, EG-018, EG-2201 and MDMB-CHMCZCA. Upon activation of CB1 by addition of the SCRAs, the activity of the functionally complemented luciferase was monitored by a 96-well luminometer. For quantification of the ligand bias, data were normalized to the efficacy of CP55,940 (arbitrarily set as 100%), which served as the ‘balanced’ reference compound within this study. Finally, intrinsic relative activities were calculated from the efficacy (Emax) and potency (EC50) values, to quantify biased agonism.

Results
Most of the selected SCRAs (e.g. SF-APINACA, CUMYL-PEGACLONE, among others) displayed a preferred signaling through the β-arrestin2 pathway, whereas MMB-CHMICA could serve as a potential ‘balanced’ agonist as it showed no difference in the EC50 nor Emax values in both bio-assays. Interestingly, EG-018 was the only SCRA showing a significant (10-fold) preference towards G protein over β-arrestin2 recruitment. A potential role for the carbazole core of EG-018 in imposing a bias towards mini-Gai was confirmed by two other carbazole-core-containing compounds, EG-2201 and MDMB-CHMCZCA.

Discussion
In a panel of 21 SCRAs with a broad variation in their chemical structure, distinct, although often subtle, preferences towards specific signaling pathways were observed. It is currently unclear what this exactly means in terms of abuse potential and/or toxicity.

Conclusions
The CB1 activation bio-assays allow the observation of biased agonism among SCRAs in the same cellular context and using the same functional assay. Therefore, these in vitro cell-based techniques, which are based on the recruitment of mini Gai-protein or β-arrestin2, can be applied to screen for the occurrence of biased agonism of a broad panel of SCRAs. This might allow a better insight into the structure-‘functional’ activity-relationship of these compounds, which is essential for a better understanding of the exact mechanism of action of these compounds and which may aid the development of new therapeutic compounds with less unwanted psychoactive effects.
Abstract ID 245
Application of a mu opioid receptor bioassay platform to study biased signalling of novel synthetic opioids.
Christophe Stove
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Aim
Fentanyl and morphine are agonists of the Mu opioid receptor (MOR), which is a member of the G protein coupled receptor (GPCR) family. Their analgesic effects are associated with unwanted side effects such as tolerance and dependence. While, on a signalling level downstream from MOR, analgesia is mainly mediated through the G protein pathway, the undesirable effects of opioids have been linked to the β-arrestin pathway. Little is known about a potential ‘bias’ (i.e. the preferential activation of one pathway over the other) of the new synthetic opioids -including fentanyl analogs- that have emerged on the illegal drug market. We have therefore developed a novel, robust bioassay platform to study the activity of synthetic opioids through both the G protein and β-arrestin pathways, in order to evaluate to what extent these MOR agonists show biased signalling.

Methods
The bioassays are based on functional complementation of two split fragments of Nanoluciferase (LgBiT and SmBiT), either fused to the receptor or to a cytosolic protein that is recruited to the receptor upon activation. These cytosolic proteins are either a mini G protein (GTPase subunit of Gα subunit as a measure for the G protein pathway) or β-arrestin2. A stable HEK293T cell line for receptor activation leading to β-arrestin2 was already available (Cannaert et al., Clin Chem, 2018); a new stable cell line for receptor activation leading to mini Gi was set up. These assays were used to evaluate a panel of 10 fentanyl analogs (fentanyl, acetylfentanyl, valerylfentanyl, tetrahydrofuranylfentanyl, ocfentanil, methoxyacetylfentanyl, cyclopropylfentanyl, cyclopentylfentanyl and crotonylfentanyl) in a 96-well plate format. Concentration-response curves were generated to yield Emax and EC50 values that were finally used to calculate the ligand bias.

Results
In the assay set-ups, MOR fused to LgBiT is combined with either β-arrestin2 or mini Gi fused to SmBiT. All tested fentanyl analogs demonstrated a concentration-dependent response at MOR in both bioassays. Only one of the ten ligands, valerylfentanyl showed a slight tendency towards the G-protein pathway when compared to the unbiased ligand, hydromorphone. On the other hand, fentanyl was found to be more biased towards the β-arrestin, in line with literature. Crotonylfentanyl, ocfentanil, acetylfentanyl, tetrahydrofuranylfentanyl, tetramethylcyclopropylfentanyl, methoxyacetylfentanyl, cyclopropylfentanyl and cyclopentylfentanyl showed no preference towards either of the pathways and hence, can be considered unbiased ligands.

Discussion
The present study reports the applicability of the bioassay, based on the recruitment of the G protein and β-arrestin2, to assess the ligand-mediated functional selectivity at the mu opioid receptor.

Conclusions
The developed bioassays allow to gain insight into the signalling of synthetic opioids, which may eventually help to better understand why certain opioids are associated with higher toxicity. The knowledge that is gained via these bioassays could possibly also help to develop novel ligands devoid of side effects, with a better therapeutic profile.
Abstract ID 247
SherLOK: Using life-style markers for perpetrator profiling through untargeted screening of hair.
Ana Miguel Pego
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Aim
There is a risk of crimes remaining unsolved when no matching DNA or fingerprints are found. If this is the case, forensic investigations focus on providing information regarding the unknown perpetrator and/or victim, this is also the case for a missing person. A rather commonly found biological matrix in crime scenes is hair. Could hair be used to construct a profile based on lifestyle markers such as one’s characteristics and habits? And could such a profile aid the tactical investigation to find and identify the perpetrator? The SherLOK project is aiming to build an analytical method capable of determining a marker set of relevant metabolites and components in hair. In order to find potential markers, an untargeted approach is developed to select and identify statistically significant features. In addition to the typical (crime related) drugs, medicines and related metabolites studied in forensic hair analysis, lifestyle markers could assist in narrowing down potential perpetrators. For such markers to be useful, they should provide relevant classification power for individuals. Interestingly, from a forensic perspective these compounds should not be too common (everybody remains a potential perpetrator) but also not too rare (no perpetrator will ever have this feature).

Methods
A total of 80 hair samples have been collected at several hairdresser shops around the city of Breda with varying price ranges and neighborhoods to ensure sample representativeness. To increase the contact surface, the samples were crushed using a 6775-freezer mill (SPEX Sample Prep). Each milled sample of 50mg in weight was then extracted overnight in 2mL of methanol (MeOH) at 50°C without any previous washing step. The next day, the MeOH has been withdrawn into a new Eppendorf tube and dried down under vacuum. The residue was then re-constituted with 200uL of 50/50 (v/v%) of mobile phase A (water + 0.1% formic acid)/mobile phase B (acetonitrile + 0.1% formic acid). Analysis was carried out by injecting 10uL of the re-constituted sample on an Orbitrap Q Exactive Focus HR/MS (Thermo Scientific) employing both reversed phase and HILIC columns in order to cover a wide range of substances. The data was acquired using XCalibur, deconvoluted (MS Convert, ProteoWizard) and uploaded to XCMS online (Scripps Research Institute) for subsequent statistical analysis and preliminary compound identification.

Results
From the total amount of samples collected, a rather similar percentage of women and men volunteered, 59 and 41%, respectively. As for smoking habits, larger disparities were found with only 15% of the subjects reporting to be smokers. With respect to alcohol consumption 12% of the individuals reported to drink daily, 45% weekly and 30% monthly whereas 15% of the volunteers indicated not to consume alcohol. Considering the age ranges of the subjects, the majority (59%) was between 18-40 years-old, followed by 28% in the 40-65 years age range and finally 13% was older than 65 years.

A smaller collection of 28 samples has been used as a learning data set. By using XCMS online, and tools such as metabolomic cloud plot, we have identified over 100 features to be statistically significant (p-value<0.01) for male-female classification. As XCMS online is paired with the METLIN database, 93 potential compound matches have been found. A difference in intensity could also be observed between the male and female data sets. An example of a compound found in the datasets analyzed was tentatively identified as piperlongumine, an alkaloid from the roots of piper longum, found in herbs and spices which was found to be 2.7 times more intense in men than women (p-value of 0.004).

Discussion
Further evaluation is ongoing to establish a suitable marker set taking into account feature classification potential, prevalence in the Netherlands and relevance with respect to typical perpetrator profiles. The first results in the SherLOK project do illustrate the potential to perform unknown donor profiling through the analysis of life style markers in hair. However, the definitive selection of potential markers requires further analysis and unequivocal identification of such substances by the use of certified standards.

Conclusions
Once a definitive selection and correct identification of the significant features have been done, a range of relevant markers contributing to the construction of a lifestyle profile will be validated and tested for use in forensic investigations.
Abstract ID 250
Development and validation of a GC-MS methodology for the determination of opioids in whole blood and pericardial fluid: Application to authentic specimens.
Cláudia Margalho
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Aim
According to the World Drug Report of 2018, opioids are not the most widely used drug globally, although they are responsible for about 76 percent of deaths, related to drug use disorders. In the European report on Drugs (2018) is referenced the abusive consumption of methadone, fentanyl, codeine, morphine, oxycodone, buprenorphine and tramadol. Thus, analysing the reports, it is verified the increasing of the illicit consumption of oxycodone, and fentanyl, making it crucial to invest in the development of analytical methodologies for its determination in several biological matrices. Although blood is the most commonly used matrix, it is important to have an alternative one, such as pericardial fluid. It may provide additional information on exposure to the substances being studied, or even make the toxicological analysis feasible when it is impossible to collect blood. Therefore, the aim of this work was to validate a method for the simultaneous determination of codeine, morphine, 6-acetylmorphine, 6-acetylcodeine, oxycodone, oxymorphone and fentanyl in whole blood and pericardial fluid.

Methods
To isolate the substances from the matrices (whole blood and pericardial fluid), volumes of 250μL of samples were subjected to a precipitation with ice-cold acetonitrile, followed by centrifugation (3000 x g for 10 min) and a fast microwave-assisted derivatization (30 sec) in a solution of 1% aqueous hydroxylamine hydrochloride and PBS (1:2, v/v). Then, the compounds were extracted by solid phase extraction using mixed-mode cartridges. A MSTFA+5%TMCS solution, was used, for a final fast derivatization of the dry extracts by microwaves during 100 sec. Codeine-D3, morphine-D3, 6-acetylmorphine-D3 and fentanyl-D5 were used as internal standards. The chromatographic separation was performed by a 7890B GC/MS 5977A, (Agilent Technologies) operated in electron impact and selected ion monitoring mode.

Results
The validation of the analytical method followed the accepted international guidelines of the Scientific Working Group for Forensic Toxicology (SWGTOX). The method was linear over a concentration range of 5-1000 ng/mL for all analytes with determination coefficients (r2) higher than 0.99 and the calibrators’ accuracy within ±20%. The limit of detection varies slightly between 3 and 4ng/mL depending on the substance and matrix. Acceptance criteria for Intra- and intermediate precision (< 20%) and accuracy (±20%) were met for all compounds in both matrices. No carryover was detected, and no interferences were found with impact on the ability to detect, identify and quantify the target analytes. The stability of processed samples in the autosampler was guaranteed for at least 24h for all the substances. The substances demonstrated stability in the matrix for 4h at room temperature and after the 3 freeze/thaw cycles for at least 8 days. The study of the dilution integrity had no significant impact on the accuracy and bias of the method. The method was successfully applied to real positive samples.

Discussion
Oxycodone and oxymorphone are keto-opioids containing a C-6 substitution of the hydroxyl group by a keto group. Numerous derivatives may occur during the formation of TMS derivatives, due to their susceptibility to keto-enol tautomerization. If the keto- opioids are hydroxylamine-derivatized prior to the trimethylsilylation with MSTFA+5%TMCS, the oxime-derivative groups are formed preventing the enolization and increasing the chromatographic resolution. The sample pre-treatment with ice-cold acetonitrile is an important experimental step in the removal of endogenous substances, such as proteins, and allows the hydroxylamine-derivatization to be performed by microwaves prior to the extraction procedure. Comparing pre-treatment with ice-cold acetonitrile or PBS, for non-keto-opioids, it was observed an increase of the ions abundancies when samples are subjected to a protein precipitation with ice-cold acetonitrile. The developed methodology has been applied to 44 whole blood samples - 11 cases were positive for codeine (5.8-54.8 ng/mL), 33 for morphine (4.2-386.0 ng/mL), 4 to 6-acetylmorphine (4.6-13.2 ng/mL) and 3 to fentanyl (4.4-45.2 ng/mL) – and to 33 pericardial fluid samples - 1 single case was positive to codeine (50.2 ng/mL), morphine (540.4 ng/mL) and 6-acetylmorphine (5.3 ng/mL).

Conclusions
This study demonstrates the improvement of the extractive efficiency, by the pre-treatment of the samples with ice-cold acetonitrile, followed by the hydroxylamine-derivatization, which allowed to reach lower limits of detection and improved the chromatographic resolution of the substances, namely the keto-opioids oxycodone and oxymorphone. Pericardial fluid may be an appropriate alternative biological specimen to blood, for the analysis of the studied drugs, to provide additional information in a forensic toxicology context. To our knowledge, this is the first GC-MS method with microwave fast derivatization that allows simultaneous determination of these compounds in postmortem biological fluids.
Abstract ID 271
Cocaine, benzoylecgonine and cuscohygrine profile in plasma from a volunteer who chews coca leaves.

Preliminary study.

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Aim
Oral use of cocaine (coca leaves chewing) is not the most common way of cocaine consumption; however, this practice is widespread in some South American countries such as Argentina, Bolivia, and Peru. Coca leaves are chewed (either alone or with alkaline substances as sodium bicarbonate or ashes) or they are brewed (coca tea) or used for preparing cookies, candies, liquors, etc. The aim of this preliminary study was the assessment of concentration-time profiles in plasma for cocaine (COC), benzoylecgonine (BE), and cuscohygrine (CUS) after coca leaves chewing (a volunteer who chewed 8 g of coca leaves). Literature regarding the metabolic profile and pharmacokinetics of oral cocaine is scarce, although CUS has been proposed as a marker of coca leaves chewing in urine, hair and oral fluid. The current study is the first one that tries to characterize the bioavailability of CUS in plasma and offers new data on the metabolic profile of COC and BE when consuming oral cocaine (coca leaves chewing).

Methods
A healthy volunteer chewed 8 g of fresh coca leaves together with an alkaline substance (sodium bicarbonate) for approximately 3 h (corresponding to 36 mg of cocaine). An intravenous catheter was inserted into the antecubital vein for blood collection. A blood blank sample was taken at the beginning of the experiment (-0.15 h). Coca leaves were then placed in the mouth at time 0 h, and blood samples were collected at 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2.25, 2.75, 3.25, 4.0, 4.5, 5.5, 6.25, 8.0, 10, 12, 14, and 24 h. Blood (4 mL) was collected into 5 mL heparinized vacutainers containing 2% (w/v) sodium fluoride. Plasma was separated by centrifugation and immediately frozen at -20°C until analysis. Plasma samples were analyzed for COC, BE and CUS by a previously validated method implying SPE and GCMS (BSTFA with 1% TMCS as a derivatizing reagent). The MS was operated in the selected ion monitoring mode. Validated parameters were LOD (10 ng/ml for COC, BE and CUS), and LOQ (20 ng/ml for COC, BE, and CUS).

Results

<table>
<thead>
<tr>
<th></th>
<th>Cmax</th>
<th>Detection window</th>
<th>Range</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>COC</td>
<td>105.8</td>
<td>1-4.5</td>
<td>d-105.8</td>
<td>46.1</td>
</tr>
<tr>
<td>BE</td>
<td>811.4</td>
<td>1-&gt;24</td>
<td>22.4-811.4</td>
<td>388.8</td>
</tr>
<tr>
<td>CUS</td>
<td>nd</td>
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Discussion
The concentration-time profile for COC showed that the concentration of COC was gradually increasing and it reached a peak at a time near 3.0 h. This profile seems different than those obtained when cocaine is administrated by intravenous, nasal or smoking via. COC has a detection window shorter than BE. BE is the major metabolite of the COC in plasma, and it is formed by hydrolysis in the mouth from COC due to the presence of alkaline substances during the chewing and also during the extraction process. The detection time of BE was more than 24 h in plasma. CUS was not detected in any plasma samples (LOD was 10 ng/mL). CUS was probably less absorbed than COC from the oral cavity at alkaline pHs (CUS has different chemical properties such as pKb and octanol partition than COC).

Conclusions
This preliminary study involves only one volunteer and definitive conclusions are therefore limited. The pattern of the use of coca leaves is variable (several times a day, or per week, or per month). In addition, people can chew coca leaves with or without alkaline substances which modifies oral fluid pH and hence the alkaloids absorption. The concentration-time profile could be therefore different in people with chronic use of coca leaves.

Probably, the gradual COC releasing in plasma does not allow to reach a high concentration in plasma and it would justify the non-occurrence of adverse effects of cocaine in people who chewing coca leaves. More research is needed for establishing a more precise concentration-time profile in plasma. The assessment of CUS in plasma samples need a more sensitive method, thus, our GCMS method for CUS detection in plasma is unable to do the discrimination between the legal or illegal use of COC in countries as Argentina, Bolivia or Peru.

Compliance with ethical standards.

The authors declare that the studies have been approved by the Comité Ético de Investigación Clínica de Galicia (registration code CEIC de Galicia 2010/372). The approved document by the CEIC de Galicia requires informed consent from all volunteers who participated in the study. The authors declare therefore that the volunteer has signed informed consent for allowing the use of the provided plasma samples in this study.
Abstract ID 281
Hydrolytic stability of 32 synthetic cannabinoids with valine- and tert-leucine methyl ester or amide as linked group in blood serum and cardiac blood samples.
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Institute of Forensic Medicine - Forensic Toxicology

Aim
The most common targets for analysis of synthetic cannabinoids in blood or serum samples are the parent compounds. Especially for synthetic cannabinoids with relatively low metabolic and/or post sampling stability, the detection of hydrolysis products could provide a proof of consumption. The aim of this study was to determine the extent of formation of hydrolysis products of 32 different synthetic cannabinoids carrying amide or methyl ester functions during storage of spiked samples under different storage conditions.

Methods
Due to the lack of commercially available standards, 32 synthetic cannabinoids were first incubated with pooled human liver microsomes (pHLMs) to determine retention times of metabolically formed hydrolysis products. Blood serum (pooled) and a cardiac blood sample were spiked with synthetic cannabinoids carrying a valine-amide, a tert-leucine-amide, a valine methyl ester or a tert-leucine methyl ester group. Four spiking mixes were prepared with each mix containing four indole and four indazole derivatives. Each mix contained a selection of derivatives preventing the formation of identical hydrolysis products. Concentrations of 1 ng/mL and 10 ng/mL of each synthetic cannabinoid were spiked to blank cardiac blood and serum samples, and stored for up to 30 days at room temperature (24 °C), in the refrigerator (8 °C) and in the freezer (-20 °C). In total, 5 sets of samples were stored to enable measurement at five different time points (0 days, 1 day, 5 days, 10 days and 30 days after spiking). A mix of deuterated internal standards was added and samples were extracted with 10 M ammonium formate and acetonitrile (1:3, v/v). After centrifugation, the supernatant was collected, evaporated to dryness and reconstituted in eluent. Parent compounds and hydrolysis products were analysed in MRM mode by LC-MS/MS.

Results
Peak areas of valine amides and tert-leucine amides were nearly stable for serum and cardiac blood during the whole period of the study and all storage conditions. Furthermore, no hydrolysis products could be observed regarding these compounds. Valine methyl esters in serum samples were hydrolysed, but only to a lower extent. The parent compound could be detected during the whole period for these compounds. After 30 days, hydrolysis of the tert-leucine methyl ester compounds in serum could only be observed in serum samples spiked at 1 ng/mL and stored at room temperature. Hydrolysis products could also be detected in samples spiked at 10 ng/mL by refrigerator conditions to a lower extent. At freezer conditions almost no formation of hydrolysis products could be observed. Cardiac blood samples spiked with valine methyl ester compounds showed a significant loss of parent compound (mostly > 50%) already after one day of storage at room temperature. At 8 °C this effect could be observed after five days. After 30 days of storage the loss of parent compound was above 90% for most of the valine methyl ester compounds under these conditions. In some cases, no parent compound could be detected anymore. However, at -20 °C, no appreciable formation of hydrolysis products could be observed. For tert-leucine methyl esters the formation of hydrolysis products was much lower in general.

Discussion
Due to the lack of appropriate deuterated internal standards for the monitored analytes, absolute peak areas were compared to assess stability. Because of relatively stable peak areas and the missing detection of hydrolysis products, a degradation of investigated synthetic cannabinoids with amide functionality seems unlikely. Therefore, enzymes present in serum and cardiac blood samples do not seem to be able to catalyse hydrolysis of these compounds. The hydrolysis of compounds with a valine methyl ester moiety as linked group is clearly favoured when compared to the hydrolysis of synthetic cannabinoids containing a tert-leucine methyl ester function. On the basis of a decreasing peak area of the valine methyl ester compounds and the increasing peak area of the corresponding hydrolysis products these SCs can be regarded as rather labile. The additional methyl group of the tert-leucine methyl ester compounds seems to have a protective effect against hydrolysis, which has been observed before in metabolism studies applying pooled human liver microsomes.

Conclusions
Due to degradation processes observed for some of the compounds, analysis of post mortem blood samples should include the respective hydrolysis products, which can be useful target analytes to detect a consumption of these synthetic cannabinoids in the absence of the parent compounds. This seems to be particularly reasonable for valine methyl ester compounds and some of the tert-leucine methyl ester compounds. Hydrolysis of the amide derivatives could not be observed for the investigated compounds. The complete degradation of parent compounds observed for some synthetic cannabinoids in this study could result in false negative findings if the corresponding hydrolysis products are not monitored.
Abstract ID 289
Risk and Opportunity Assessment System - Probability and Consequences Method.
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Aim
Organizations face factors and influences, both internal and external, that make it uncertain whether and when they will achieve their goals. The effect this uncertainty has on an organization’s objectives is called “risk.” This definition, expressed in NP ISO 31000, highlights the organization’s need to promote risk management mechanisms, aiming the effectiveness’ improvement of the management system. Forensic Toxicology laboratories are no stranger to risk and typically have many procedures in place to address diverse areas, such as employee hiring/training, quality assurance, testimony expectations and method validation. The most recent normative accreditation references propagate risk-based thinking, contributing to continuous improvement, minimizing undesirable effects, and also adding an important resource for improvement opportunities analysis.

Accreditation is the independent evaluation of conformity assessment bodies (in this case Forensic Laboratories working to ISO/IEC 17025). Accreditation bodies are normally themselves peer assessed through an international system administered by the International Laboratory Accreditation Cooperation. Accreditation bodies are established in many countries with the primary purpose of ensuring that conformity assessment bodies are subject to oversight by an authoritative body.

Methods
The 2017 version of ISO/IEC 17025 General requirements for the competence of testing and calibration laboratories was recently published and contains major changes as closer interaction with ISO 9001, introduction of the need to perform an impartiality risk assessment; assessment of risks/opportunities to the operation of the laboratory and ‘Risk-based’ internal audits.

While there are many approaches to evaluating risk, ISO/IEC 17025 does not specify a particular approach, nor a formal or documented procedure. In this review we intended to provide tools for meeting the ISO/IEC 17025:2017’s risk and opportunities requirement in the context of forensic laboratories.

Results

Discussion
To be effective, risk and opportunity management, from its preparation, implementation to monitoring, should be integrated into the planning and programming cycle of the organization. Risk management implies the identification of these events when related to the organization’s objectives. Application of the Risk and Opportunity Assessment System - Probability and Consequences Method, which appears as a frequently selected tool to carry out the risk and opportunity assessment, must be approached to ensure an adequate application to the forensic laboratories.

The application of this method has as evidence the risk matrix based on the two criteria, Probabilities and Consequences. As an example of risk assessment we can focus on the possible use of two GC/HS-FIDs for ethanol determination that could contribute to obtaining divergent quantifications for a sample. The risk and likelihood would be medium and it is considered necessary to apply a control measure. In this case, a possible alternative would be to participate in an interlaboratory study for both equipment. This risk should be continuously assessed.

In fact, given the aim of simplicity inherent in this methodology, the absolute real values of risk/opportunity, probability and consequences are not used, but rather a levels’ scale of four possibilities, being the obtained information semi-quantitative and indicative.

The risk and opportunity treatment phase establishes a basis for increasing the effectiveness of the management system, achieving results and preventing negative effects taking into account the organization’s objectives (opportunities), organizational context, internal and external factors, and requirements of relevant stakeholders.

Conclusions
An adequate risk/opportunity assessment favours a proactive and not only reactive attitude of organizations in order to reduce and avoid unwanted effects in pursuit of their objectives.
Abstract ID 294

Analytical challenges in the forensic toxicological analysis of a subgroup of novel synthetic opioids, the “U-drugs”.

Maurice Wilde

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Aim

In total, nine novel synthetic opioids from the class of so-called U-drugs or Utopioids (developed by the Upjohn Company), another class of new opioids besides fentanyl analogues, have been reported to the European Monitoring Centre for Drugs and Drug Abuse (EMCDDA) until the end of 2018. The unambiguous identification of these compounds or their metabolites is often hampered by strong structural similarities (e.g. leading to isomeric metabolites). The aim of this study was to investigate the phase I in vitro metabolism of eight compounds using pooled human liver microsomes (pHLM) and to identify specific analytical targets and analytical pitfalls.

Methods

Seven U-drugs (Isopropyl-U-47700, N-Methyl-U-47931E, U-47700, U-47931E (Bromadoline), U-48800, U-49900 and U-51754 (Methene-U-47700)) and a related opioid (AH-7921) were purchased either as research chemicals from online vendors or as certified reference material. Identity and purity of research chemicals was confirmed by LC-ESI-qTOF-MS, GC-MS and NMR analysis. Each compound was incubated with pHLM to investigate the in vitro phase I metabolism. The concentrated incubates were analysed by LC-ESI-qTOF-MS in positive full scan and bbCID scan mode. DataAnalysis® software (Bruker) was used for data evaluation.

Results

Six of the investigated parent compounds form three pairs of constitutional isomers, which show the same molecular formula resulting in an identical exact mass. The fragmentation reactions observed in HRMS analysis were quite similar within this class of opioids. The bbCID spectra for the isomers Isopropyl-U-47700 and U-49900 exhibited a few differences, whereas AH-7921 and U-47700 produced quite similar bbCID spectra with mostly identical fragment ions. The pair of U-48800 and U-51754 showed identical fragment ion spectra in the MS experiments showing almost the same ion ratios for the most abundant fragment ions. All compounds showed extensive metabolism in the pHLM assay. N-Dealkylation was the most predominant metabolic reaction observed for all the investigated compounds. The resulting mono- and di-dealkylated metabolites showed isomerism to other metabolites or parent drugs within the group of investigated opioids. Hydroxylations and further oxidations reactions, such as formation of ketones or presumably carboxylic acids, were also detected, though the most frequent oxidation products were mono- and dihydroxylated metabolites. Considering the in vitro data, hydrolysis of the amide function seems to play a minor role for U-drugs. Dehalogenation only occurred for the bromine-substituted analogues in the employed assay, although the corresponding metabolite was only detected with low abundance.

Discussion

Due to the formation of unique fragment ions the isomers Isopropyl-U-47700 and U-49900 may be identified solely by mass spectrometric analysis. The isomeric pair AH-7921 and U-47700 yielded very similar bbCID spectra, and only low abundant fragment ions and their differing ion ratios may allow distinction of these isomers when relying on MRM analysis, with the drawback of a considerable loss in sensitivity. U-48800 and U-51754 only differ in the positions of the chlorine substituents of the dichlorophenyl moiety (regio-isomers). Both compounds exhibited the same fragment ion spectra with the same ion ratios, rendering a differentiation by MS analysis impossible. The extensive metabolism of the investigated compounds suggests the inclusion of metabolites as additional analytical targets for the analysis of biological specimens. The main metabolic reaction, N-dealkylation, leads to mono- and di-dealkylated metabolites, with some of them showing isomerism to each other or to some of the investigated parent compounds (e.g. N-desethyl-U-49900 and U-47700), complicating the correct identification and interpretation of analytical findings. Evaluation of unique metabolites, such as hydroxy-metabolites, and/or the development of chromatographic methods facilitating separation of isomeric compounds and their metabolites seems mandatory in these cases.

Conclusions

Sufficient chromatographic separation is essential for correct identification of U-drugs and their metabolites. The extensive metabolism of these compounds and the resulting interferences need to be considered when interpreting analytical findings in biological samples.
Abstract ID 295

New Findings on Type and Amount of Tryptamine Derivatives in the Poison of the Colorado River Toad (Incilius alvarius) using LC-HR-QToF-MS and LC-MS/MS

Merja A. Neukamm

Nicole Zimmermann1, Tobias Scholl2, Johannes Penner3, Amy Autret4, Thomas Zander2, Laura M. Huppertz1, Volker Auwärter1, Merja A. Neukamm1

1Medical Center - University of Freiburg, 2ESA-Test GmbH, 3Chair of Wildlife Ecology & Management Freiburg, 4Crime Laboratory Tucson Police Department

Aim

The poison of the Colorado River Toad (Incilius alvarius) can be smoked as a recreational drug because it contains the strong hallucinogen 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT). Herewith, we propose new approaches for the comprehensive analysis of the poison including the enrichment of compounds other than 5-MeO-DMT. Methods for LC-HR-QToF-MS and LC-MS/MS analyses to detect both known and unknown substances in the toads’ poison were developed. One aim was to use the LC-MS/MS method to compare the concentrations of different tryptamine derivatives between samples from zoo (captive kept) and wild toads, which to our knowledge has not been done before.

Methods

To collect poison samples, the paratoid glands as well as the glands on the upper and lower legs of the toads were gently squeezed (‘milking of the toads’). Samples were air-dried for several days. Three samples from toads from the zoo in Leipzig and three samples from wild toads from Arizona, USA, were used for first analyses. However, more samples will be analyzed at a later stage. Repeated extraction was performed with acetone/H2O (70/30, v/v) after homogenization using a ball mill. Enrichment of compounds other than 5-MeO-DMT was carried out using an analytical HPLC and the valve of the mass spectrometer. Qualitative analyses were performed using LC-HR-QToF-MS for screening and LC-MS/MS with MRM transitions of reference standards and hypothetical MRM transitions of further substances. Comparison of the concentrations of different tryptamine derivatives in zoo and wild toads, which to our knowledge has not been done before.

Results

5-MeO-DMT, bufotenin (5-OH-N,N-dimethyltryptamine), DMT, 5-MeO-tryptamine, 5-OH-N-methyltryptamin and tryptophan were identified in the extracts by comparison with reference standards. 5-MeO-N-methyltryptamine was tentatively identified by detection of the accurate mass and corresponding fragments in HR-QToF analysis and by signals of hypothetical MRM transitions in LC-MS/MS analysis. Moreover, at least two isomers of hydroxylated MeO-DMT were tentatively identified in the poison samples by HR-QToF-screening. The quantitative analysis showed the following approximate mean concentrations of tryptamine derivatives in zoo and wild toad poison samples in relation to the weight of the dried samples: 5-MeO-DMT 380 and 400 mg/g, bufotenin 0.53 and 5.3 mg/g, DMT 380 and 310 µg/g, 5-MeO-tryptamin 200 and 510 µg/g, 5-OH-N-methyltryptamine 54 and 340 µg/g in zoo and wild toad poison samples, respectively.

Discussion

As the standards were not available at this point, the confirmation of the presence of 5-MeO-N-methyltryptamine and hydroxylated MeO-DMT in the extracts is still pending. The exact positions of the hydroxy group and the methoxy group of the hydroxylated MeO-DMT could also not be determined. The concentration of 5-MeO-DMT of about 400 mg/g seems high in comparison to amounts between 50 and 150 mg/g estimated by Erspamer et al. (Biochemical pharmacology, 1967). In contrast to the skin extract examined in their study, we extracted dried poison, which might explain the differences. Nevertheless, the values should be confirmed by further analyses. To prove the observation that wild toad poison samples contain higher concentrations of certain tryptamine derivatives than zoo samples, more samples should be analyzed using the developed MRM method.

Conclusions

New approaches for the comprehensive analysis of the toad poison including the enrichment of compounds other than 5-MeO-DMT were proposed. Moreover, methods for LC-HR-QToF-MS and LC-MS/MS analyses were developed. Wild toad poison samples seem to contain higher amounts of bufotenin, 5-MeO-tryptamine and 5-OH-N-methyltryptamine than poison samples from zoo animals. By analyzing further poison samples from various zoos where the animals are kept under different environmental conditions, reasons for these concentration differences can be investigated. Other studies will include the vaporization of the poison with a special device to simulate the consumption process and to assess the content of tryptamine derivatives in smoke condensates by using the developed methodology.
Abstract ID 310
A non-destructive and versatile approach for the detection of psychoactive substances by X-ray powder diffraction.

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Aim
Since new psychoactive substances (NPSs) entail a serious health risk for the society, there is a high demand for sensitive analytical detection methods. However, identification of NPSs may be complicated with current analytical methods either due to the structural similarities between these compounds or some impurities in the street samples. Therefore, we investigated the potential of X-ray powder diffraction (XRPD) for the identification of drugs. XRPD instrumentation has made significant progress in time and its use brings an innovative approach to the detection of not only the main substance but it also appears to be a very useful method for the identification of either organic or inorganic cutting agents used to dilute the relevant street drug.

Methods
Each sample was applied on the silicon pod - the crystals were crushed and flattened to create a plane surface. Bruker D2 Phaser powder diffractometer (Bruker AXS, Germany) with parafocusing Bragg–Brentano geometry using CuKα radiation (λ = 1.5418 Å, U = 30 kV, I = 10 mA, room temperature) was used for collecting the XRPD data. The data were collected (5 - 60 °2θ angular range, 0.020 °2θ step size, 0.9 s per step counting time) by an ultrafast LYNXEYE XE detector and HighScore Plus 3.0e software package was employed for subsequent data evaluation.

The IR spectra of the samples were measured by the ATR technique with a diamond crystal on a FT-IR Nicolet iS50 spectrometer (Thermo Scientific, USA) with a Tungsten-halogen MIR radiation source, KBr beam splitter and DLaTGS detector. The spectra were recorded as an average of 256 scans in a spectral region of 4000–400 cm-1 with a resolution of 4 cm-1.

The Raman spectra were acquired on a DXR SmartRaman spectrometer (Thermo Scientific, USA) equipped with two lasers (excitation wavelengths 532 and 780 nm). The samples were analysed in glass vials in a spectral region of 400–3000 cm-1 with a resolution of 2.4–4.4 cm-1 and the spectra were processed with the correction of fluorescence.

Results
Two series of psychoactive substances (heroin, cocaine, mephedrone, ephylone, butylone, JWH-073 and naphyrone) were measured. The first series was seized by a Czech law enforcement agency, the second series comprised of the standards of the same compounds. Both series of samples were analysed using XRPD, and Infrared and Raman spectroscopies. Furthermore, XPRD was used for analyses of 15 seized cocaine samples with focus also on the cutting agents.

Discussion
By comparison of diffraction patterns of each seized compound with the respective standards, we showed that XRPD was able to identify each of the illicit substances in the seized samples. Infrared and Raman spectroscopies in both cases were not able to detect the compounds in all of the samples. Moreover, results from the analyses of cocaine street samples showed, that XRPD might detect not only the main compound but also cutting agents in a single run analysis. With respect to the physical basis of how the XRPD works, some signals in the diffraction pattern may overlap, however, they do not overlap entirely. Therefore, despite the overlap of some signals, in case of a not too complex mixture, the identification of compounds in the unknown sample with a proper database might be possible.

Conclusions
X-ray powder diffraction was successfully used for the identification of seven psychoactive substances and for the detection of cocaine in several street samples. These results show that XRPD could be a valuable addition to the range of analytical tools used to detect these compounds in illicit drug samples. However, yet a number of difficulties need to be overcome for future use in forensic practice.

This study was funded by a specific university research (project MSMT No. 21-SVV/2019), by the Ministry of Interior of the Czech Republic (project V10172020056) and by the Ministry of Industry and Trade (FV30483).
Abstract ID 312
Drugs of Abuse Screening in Urine and Quantification from Whole Blood using PaperSpray Technology for Forensic Use.
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Thermo Fisher Scientific

Aim
PaperSpray is a rapid analysis technique which is particularly beneficial for the analysis of compounds in biological matrices, such as blood or urine. Because no sample preparation is required and analysis times are as short as 1-2 minutes, the technique offers a strong advantage over traditional methods which rely on chromatographic separation with their associated solvent use and time required for samples preparation and analysis. Of particular interest in the forensic community are screening assays, which require short turnaround times and the ability to screen for multiple compounds in a single run. Here we present a method for rapid screening of 20 drugs of abuse directly from dried urine spots. We then developed a one minute analytical method by paper spray-mass spectrometry that detects EDDP, a primary opioid metabolite of methadone, that eliminates the need for sample preparation and enables quick turnaround times needed for routine compliance testing.

Methods
For the screening method, a total of 20 compounds (opiates, amphetamines, cocaine and PCP) were spiked into donor urine. Isotopically labelled internal standards were added. Four concentration levels were prepared: at cutoff, 2 times higher than cutoff, 5 times lower than cutoff, and blank. Eight microliters of each respective urine sample was spotted onto a paper spray plate for analysis. The spray solvent was 90% acetonitrile, 10% water, and 0.01 % acetic acid. Data was acquired for 1 minute per sample, and each concentration level was measured 5 times for screening. For the quantification method, EDDP was spiked into human whole blood at various concentrations to yield a nine point calibration curve ranging from 1.75 to 500 ng/mL and three quality control (QC) samples at concentrations of 50, 100, and 200 ng/mL. A total of 240 samples, consisting of either calibrators or quality control samples, were spotted onto the paper plates at a sample volume of 8 µL each and were oven dried for 30 minutes at a temperature of 50 °C. Sample plates were then loaded onto the Thermo Scientific™ VeriSpray™ plate loader, which holds up to 10 paper spray plates (24 paper strips per plate), and connected to a VeriSpray ion source and a Thermo Scientific™ TSQ Quantis mass spectrometer. The chronograms for both EDDP and d3-EDDP from 0.1 to 0.95 minutes were integrated and area counts were used to quantitate each compound. Total mass spectrometer run time was one minute.

Results
All 20 compounds met their respective screening cutoff levels (≤15% RSD of response ratio at the cutoff level, and the absolute Area Under the Curve (AUC) at least 4x higher than the AUC of the matrix blank). The lower limit of quantification (LLOQ) for the TSQ Quantis for EDDP was 3.5 ng/mL and was defined as the lowest calibration standard analyzed that yielded < 20 % accuracy and < 15 % CV for 9 replicate injections. The labeled d3-EDDP present in all 240 samples was monitored for the entire run and had a peak area RSD of 32% spanning the entire run and a RSD of 1.5 % when comparing the peak area ratio of the labeled and unlabeled EDDP standard at 50 ppb level.

Discussion
A method for rapid drugs of abuse screening for forensic use was developed using the Thermo Scientific™ new VeriSpray™ PaperSpray ion source. Without any sample preparation, 20 compounds, including opiates, amphetamines, cocaine, and PCP, were screened from urine within 1 minute and met precision and accuracy requirements at the respective cutoff level. Calibration curves and QC samples were acquired for EDDP in whole blood to confirm and quantify this compound previously screened in urine. The method in whole blood was also one minute per sample and after a total of 240 automated repeated injections yielded robust, quantitative analysis of EDDP by a triple quadrupole mass spectrometer.

Conclusions
PaperSpray technology can be used as a rapid screening method for drugs of abuse directly from dried urine spots. It can also be further applied for the confirmation and quantification in whole blood. No sample preparation other than spiking an internal standard and spotting the urine or whole blood samples on the paper is required. A total method length (analysis time + automated sample handling time) of 1.5 minutes makes it a highly efficient and cost effective screening and/or quantification method. The method proved to be reproducible over 240 injections without any significant loss in signal, which makes it appropriate for a routine analytical method.
Abstract ID 313
UPLC-MS/MS analysis of illicit drugs in wastewater in the city of Lisbon and Almada between 2015 – 2018. Cross correlations with major nicotine biomarker.

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Aim
Wastewater analysis is a rapidly developing scientific discipline with potential for monitoring real-time data on geographical and temporal trends in illicit drug use. The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) has been supporting all the initiatives that may lead to a better and more precise knowledge of the drug abuse problem. The Sewage Analysis Core group Europe (SCORE) and the ES1307 COST action (Sewage Biomarkers Analysis for Community Health Assessment emerged as promising networks and wastewater (WW) analytical tools, dealing with a different and quicker approach for obtaining estimates of drug abuse at national and European levels. Two major Sewage Treatment Plants (STP) in the region of Lisbon (Lisbon-Alcântara and Almada-Mutela) have been regularly participating in the SCORE studies since 2014 contributing with qualitative and quantitative data on the major drugs and metabolites under study in a total of 15 drug biomarkers. The detection of drug residues (unaltered drug or drug’s main metabolites) requires analytical techniques with high selectivity and specificity such as the LC-MS/MS. In this paper, we present an UPLC-MS/MS method developed for detection and quantification of illicit drugs in wastewater influents, as well as the revelation of interesting correlations among sewage drug levels.

Methods
Between 2015 and 2018, in the framework of the SCORE project, 78 samples of two STPs in the region of Lisbon were analyzed. The UPLC-MS/MS method and sample preparation procedures were developed and validated, for the quantitative analysis of Morphine, Cotinine, 6MAM, MDA, MDMA, Amphetamine, Methamphetamine, Mephedrone, Ketamine, Benzoylcegonine, Cocaine, EDDP, Methadone and THC-COOH, with a recovery range from 47% to 105% and LOQ values in the range of 1 to 10 ng/mL.

Results
The prevalence of the substances in the WW samples analysed were: Morphine, Cotinine, Benzoylcegonine, Cocaine, EDDP, Methadone and THC-COOH (100%); MDMA, Amphetamine, Methamphetamine (97%); Cocaethylene (88%); 6MAM (65%); Ketamine (22%); Mephedrone (29%) and MDA (1,2%). Correlations were observed among sewage drug levels between tobacco use (cotinine) and morphine (opiate source) or cocaine (benzoylcegonine), expressed as amounts of these biomarkers per thousand inhabitants.

Discussion
The correlations considered were the relationship between variables used during the full week studies in 4 years (2015-2018) in all EMCDDA/SCORE reports, cotinine/benzoylecgonine, cotinine/morphine, cotinine/MDMA, expressed per 1000 inhabitants. It was found systematic positive (and statistically significant) correlations between most of these drug concentrations shifts along the week studies in every year in both STP. When cotinine concentration increases (or decreases) morphine (or some other times benzoylecgonine also) increase, or decreases. Cotinine/MDMA levels either have no correlation at all or show an inverse correlation. These consistent yearly and local relationships constitute to our knowledge a major finding in sewage drug profiles opening discussions about the reasons that underlie these ties. Also they are in line with previous published data from one of the authors.

Conclusions
The UPLC-MS/MS method developed showed adequate levels of performance to monitor illicit drugs in wastewater samples. The consistent findings of some correlations among some biomarkers seem to confirm previous research and published work. We also emphasize the importance of plotting daily profiles of several drugs simultaneously by opposed to singular drug plots, as common practice on the presentations of EMCDDA/SCORE data. This approach will provide a better comparison of local drug use profiles on all temporal studies (day of the week and year).
Abstract ID 317
Method development for the identification of several mycotoxins by laser desorption – mass spectrometry (LDTD-Orbitrap).

**Estelle Flament**

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**Aim**

We present a new analytical technology, the Laser Diode Thermal Desorption (LDTD) and its application for the identification of several mycotoxins in fungi. Molecules are orellanin (exact mass 252,03769 uma): a highly nephrotoxic molecule; muscarin (exact mass 174,14886 uma): a neurotoxic molecule not reaching the central nervous system; ibotenic acid and muscimol (exact mass 158,03221 uma and 114,04238 uma respectively): neurotoxic molecules reaching the central nervous system. These toxins are annually responsible of poisoning cases or even cases of death. The aim of this application is to identify these toxins in mushrooms or in residual meal.

**Methods**

This method of identification relies on the deposit of a volume of standard solution of each molecule mixed with a solution of muscarin-d9 on a LazWellTM plate (Phytronix), then dried under water-free air flow and analysed by Laser Diode Thermal Desorption or LDTD (Phytronix) coupled with a high resolution mass spectrometer, QExactive technology (Thermo Scientific). The acquisition in SIM or PRM mode lasts 0,17 minutes. The determination of the LOD of each molecule was done with a standard solution deposit. Finally, the fragmentation of each molecule with the LDTD technology (ionization using a Corona needle) was compared to the ESI ionization fragmentation. This method of identification was tested on some real mushroom samples: a piece of the cap of an Amanita muscaria (mushroom containing ibotenic acid and muscimol) and a Macrolepiota procera (mushroom free of interest molecules), previously dried, were ground and ultrasonicated with water. A volume of 8 µL of the supernatant was dropped into the LazWellTM.

**Results**

The 5 molecules were formally identified using their exact mass. It should be noted that the retention time does not exist with the LDTD technology. The only way to identify a molecule is its exact mass. The LOD values with a standard solution deposit are: orellanin: 250 pg/8µL, muscimol: 25 pg/8µL, ibotenic acid: 250 pg/8µL, muscarin: 6,25 pg/8µL. For the fragmentation of the molecule, daughter ions were the same with Corona needle as ESI ionization for muscimol and muscarin. Molecules of interest were not found in the cap of the Macrolepiota procera and the presence of muscimol in the cap of A. muscaria was confirmed.

**Discussion**

**Conclusions**

We have developed a method for the rapid determination of several mycotoxins (orellanin, muscimol, ibotenic acid and muscarin) with a LDTD-HRMS/MS technology. The interest of this technology is to provide immediate elements of confirmation and assessment of the severity of the intoxication in order to propose the best therapeutic treatment as soon as possible. This notion of rapidity is essential in a context of fungal poisoning because some treatments such as gastric lavage become useless and ineffective within a delay too far from the ingestion. This technique will be adapted to the detection of other mycotoxins and other matrices and be compared to conventional technology such as UHPLC-HRMS/MS.
Abstract ID 325
In search of psychoactive THC in cannabis-based dietary supplements.

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Aim
Cannabis sativa has been used since ancient times for its medicinal and psychoactive properties. Recent research has indeed revealed its biological potential in the treatment of neurological diseases, which lead to a recent boom of cannabis-based products, often marketed as oral dietary supplements. Along with high contents of the main active ingredient, cannabidiol (CBD), such products may contain low amounts of psychoactive tetrahydrocannabinol (THC) and its presence has to be determined before they enter the market.

The aim of this work was to develop a fast and simple method for the determination of CBD, THC and their precursors in a wide range of products, including edible oils, hydrophilic extracts, capsules and dried plant material.

Methods
The main goal was to determine both high concentration of CBD and low amount of THC in one single analytical run. This was achieved by combining UHPLC method with DAD and MS/MS. By using two detectors differing in sensitivity, we were able to significantly expand the detected concentration range without the need of a extra sample dilution and re-analysis. The resulting method included a simple dissolution or extraction, depending on the type and texture of the sample: Hydrophilic samples were dissolved in isopropanol, hydrophobic samples and plant material were extracted by a mixture of isopropanol:ethylacetate (1:1 v/v). All resulting extracts were further diluted into mobile phase and analysed by reversed-phase chromatography with Poroshell 120 EC-C18 column, DAD detection at 235 nm and MS analysis in a positive electrospray mode using multiple reaction monitoring (MRM) technique.

Results
By using a simple dilution as a pre-treatment method we could analyse a wide range of different samples in one batch, and our method was suitable even in commercial environment as we reached an acceptable analysis time (8 min per sample) and a suitable limit of quantification (2-4 μg/g). The final method was further validated for accuracy, precision and matrix effect and all validation criteria were met. Finally, the resulting method was applied for the determination of major cannabinoids in real samples of oral supplements, manufactured both by local and international producers, and which were about to be introduced on the market in the Czech Republic, Germany and Spain. Samples included capsules, edible oils and extracts marketed as dietary supplements. Both THC-negative and THC-positive samples were found among the analysed products.

Discussion
In comparison to other methods for analysis of cannabinoids in consumer products, we were able to determine both low concentrations of THC (above 4 μg/g) up to high concentrations of CBD (up to 40 mg/g) in one single analytical run. The method can be therefore used in a commercial setting, especially when analysing different types of products with a wide range of analyte concentrations.

Conclusions
Abstract ID 338  
Determination of Synthetic Cannabinoids Usage Frequency in Probationers.  
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Aim  
Synthetic cannabinoids are a group of psychoactive compounds that mimic the effects of Δ9-tetrahydrocannabinol, the primary psychoactive constituent of marijuana (Cannabis sativa L). Legal alternatives to traditional drugs of abuse, such as the synthetic cannabinoids, have been gaining popularity over the last several years.  

Due to cannabis users partly shift to the consumption of 'herbal mixtures' as a legal alternative to cannabis products in order to circumvent drug testing, the analysis of synthetic cannabinoids in biological matrices becomes more important in the fields of forensic and clinical toxicology. Moreover, comprehensive methods covering the majority of synthetic cannabinoids already identified on the drug market are still lacking.  

Therefore, the aim of this study was to develop a sensitive analytical method for the determination of 56 synthetic cannabinoids and their metabolites in urine samples of probationer.  

Methods  
The samples were collected from the probationers who came to Institute on Drug Abuse, Toxicology and Pharmaceutical Science of Izmir Ege University between (April 2017-June 2017). Considering the large number of samples that send to the institute, systematic sampling was performed and 500 urine samples were taken from showed negative results in pre-screening tests. Urine samples were extracted via liquid–liquid extraction. In this study, 200 ml of 2M potassium carbonate (K2CO3) buffer (pH: 7-7.5), 2 ml of chlorobutane and JWH-073-d7 (1 ng / ml, IS) were added to 1 ml of urine. The identification was performed by liquid chromatography–tandem mass spectrometry (Shimadzu 8040).  

Results  
Despite the broad range of physicochemical properties of the synthetic cannabinoids, method allowed acceptable recoveries for all the studied compounds at 4 different concentration levels. For the lowest concentration of 0.1 ng/ml, the recovery was between 71.5% and 97.7%. According to our results, synthetic cannabinoids were positive in 72 of 500 urine samples (14.4 %). The most frequently detected synthetic cannabinoid was 5F-NPB-22 (46 %). The use of 5F-NPB-22 in Turkey has increased rapidly recent years. (S)-AB-FUBINACA, 5F-ADBICA, AM-2201 and metabolites, JWH-122 and metabolites were also detected in urine.  

Discussion  
It is known that synthetic cannabinoids are popular among probationers for avoiding legal processes due to their rapidly changing nature and the difficulty of detection in pre-screening tests. With this study, a significant positive result was found with high-sensitive and wide-ranging scans in samples that had negative results in the pre-screening test. Therefore, comprehensive chromatographic analyzes are required to avoid false negative results.  

Conclusions  

Abstract ID 341
Differentiation of blood spots originating from uniovular twins based on xenobiotics analysis.
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Aim
The fact that uniovular twins possess identical genetic material is one of the dogmas of genetics. Although the results of metagenomic analyzes indicate slight differences in twins genomes, they have not been used yet in routine forensic analysis.

Methods
We present a case study of a suicide or the murder and suicide of two male uniovular twins. During investigation, a knife was secured with revealed blood spots on the blade and hilt with ageneric profile corresponding to the genetic profile of twins, and blood spots secured on clothing. Extraction of xenobiotics from the blood and blood spots was carried out using the liquid/liquid method. The study was carried out in the range of 521 psychoactive compounds, drugs and their residues using LC-MS/MS. The analysis was conducted by following two MRM pairs on a Sciex 5500 QTRAP mass spectrometer operated in ESI mode coupled with an liquid chromatograph (2x ExionLC AC Pump, ExionLCDegaser, ExionLCautosampler, AC ExionLC Column Oven) on a Kinetex C18 100 x 3 mm, 2.6μm chromatografic column using a methanol/water mobile phase system.

Results
Amphetamine was found in the blood of one of the men at a concentration of 386.5 ng/ml while THC and THCCOOH were found in the blood of the second at concentrations of 7.2 ng/ml and 85.1 ng/ml, respectively. The presence of amphetamine was found on the blade of the knife, while THC and THCCOOH were found on the knife handle. Amphetamine or THC and THCCOOH or amphetamine and THC and THCCOOH were found in clothing spots.

Discussion
The cause of death were stab wounds. The presence of various psychoactive compounds in the blood of identical twins was the reason for trying to analyze blood spots on the basis of xenobiotics. The obtained results allowed for assigning blood spots to the appropriate one from twins. Stains in which only amphetamine was found came from the first of the twins. However, those in which THC and THCCOOH were detected originated from the other one. Samples in which both amphetamine and THC and THCCOOH were found were from both men.

Conclusions
The results of the study show that the use of sensitive analysis of xenobiotics in blood spots may in some cases allow for the differentiation of blood traces from uniovular twins.
Abstract ID 352
Analytical workflow in the era of the opioids crisis: screening for novel synthetic opioids.

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Aim
The opioids crisis has been a fixture in North American forensic toxicology laboratories for several years now, and shows no sign of slowing down. In 2017, 3 990 deaths related to opioids were recorded in Canada, with fentanyl and novel synthetic opioids (NSO) involved in 72% of cases.

NSO, a subcategory of novel psychoactive substances (NPS), display a number of characteristics rendering analytical identification challenging. Indeed, these are highly structurally similar compounds with high potency and therefore low biological concentrations. Moreover, new analogs are constantly emerging, making it hard for certified reference materials manufacturers and hence forensic toxicology laboratories to keep up.

To tackle these challenges and put an end to the multiplication of method targeting only a handful of NSO, a comprehensive, flexible and responsive screening method was developed and validated.

Methods
Biological matrices were extracted by protein precipitation, where 100 µL of blood or urine was mixed with 10 µL of an internal standard solution (fentanyl-D₅ 0.1 µg/mL), and diluted with 100 µL of MeOH:0.2% formic acid (50:50 v:v). Precipitation was achieved by adding 400 µL of an acetonitrile:acetone (70:30 v:v) solution and vortexing for 5 minutes. Samples were centrifuged and 200 µL of the supernatant was diluted with 50 µL of 1.5% formic acid. The extract was injected on an Agilent 1200 HPLC coupled to a Sciex 5500 QTRAP operated in positive electrospray ionization mode with multiple reaction monitoring (MRM). The chromatographic column, an Agilent Eclipse Plus C18 (2.1 x 100 mm, 3.5 µm) was held at 50°C throughout the 13 minutes analysis, including washing and equilibration time. A gradient from 10 mM ammonium formate:MeOH (98:2 v:v, pH 3) to acetonitrile was applied at a 650 µL/min flow, aiming to achieve baseline resolution of the numerous NSO isomers targeted.

Results
Validation of this identification qualitative method covering 54 NSO compounds was carried out under the SWGTOX, CAN-P-1578 and ISO 17025:2017 requirements. Limits of detection (LOD) were estimated for each compound by analyzing blood samples spiked at decreasing concentration. Most analytes (n = 40) exhibited an LOD ≤ 0.1 ng/mL. The presence of carryover after a high concentration (200 ng/mL) blood extract was monitored, with only cis-3-methyl norfentanyl being problematic. Specificity was confirmed by the analysis of 15 blank blood and 15 blank urine specimens (antemortem and postmortem), 80% of which demonstrated no interference. No significant interference was detected from any of the 175 exogenous compounds analyzed.

Discussion
With several reports of sub-2 ng/mL concentrations for NSO in both antemortem and postmortem cases, the developed method had to demonstrate low LODs for all compounds, which was successfully achieved here. The baseline resolution challenge was also met for this starting set of 54 analytes, except for the two metabolites butyryl norfentanyl and isobutyryl that do coelute.

This method was added to the standard testing panel applied to all incoming samples. Since the same extraction procedure is used for cannabinoids analysis, NSO testing is minimally disruptive to the analytical workflow in place and thus highly efficient. Where quantification of positive findings is deemed necessary, it is carried out via standard addition in-house or by an accredited external laboratory.

The general unknown screening (GUS) methods by gas chromatography/mass spectrometry (GC-MS) and liquid chromatography/quadrupole time of flight (LC-QToF) act as the early warning systems, detecting the presence of a new NSO in case work albeit imperfectly due to their higher LODs. Once a novel compound enters the case work, it can be added to the identification qualitative LC-MS/MS method developed after a limited validation in one experiment requiring less than 50 injections only. Substances which seem to be integrating to a larger extent the drug market, requiring a large number of quantification analyses, can be integrated to the permanent quantitative LC-MS/MS method. On the other hand, compounds in the method which have not been seen over the past year can be dropped; their potential reappearance to be picked up by the GUS methods and re-introduced in the method with no necessary validation work. Since the implementation of this method as a standard screening tool, NSO detection has increased, demonstrating its usefulness and importance in the current setting.

Conclusions
The ever increasing role of NSO in the North American opioids crisis calls for a different approach, global yet responsive. By allowing dynamic compound addition and removal with minimal work, this method initially validated for 54 NSO achieves this goal. Given the success obtained with NSO, other NPS such as designer benzodiazepines will also be integrated in the future.

Properly assessing the prevalence of NSO is relevant not only to individual postmortem or driving under the influence of drugs cases, but also to provide an accurate picture of drug consumption habits and properly orient public safety policies. The forensic toxicology laboratory is uniquely placed to do so, but must put in place the proper methods.
Abstract ID 356

Alicyclic fentanyls’ µ-opioid receptor activation changes from potent full agonists to partial agonists with increasing ring structure.

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Aim

Alicyclic fentanyls’ share their overall chemical structure with fentanyl except for the substitution of the propionamide with amides containing increasingly large cyclic structures containing either a cyclopropyl, cyclobutyl-, cyclopentyl-, cyclohexyl- or 2,2,3,3-tetramethylcyclopropyl ring. Both fentanyl and fentanyl analogs are known to activate the µ-opioid receptor to various extent depending on their exact structure. Investigating structurally related analogs allows for structure-activity comparisons. Therefore, we aimed to determine the alicyclic fentanyls’ µ-opioid receptor activity and investigate any structure-activity relationship.

Methods

The Perkin-Elmer AequoScreen recombinant CHO-K1 cell line expressing the human µ-opioid receptor was used to perform a dose-response activity-assay. The cells were cultured to a confluency of 70-90 %, passaged, centrifuged and resuspended in pre-warmed assay media (DMEM/Ham’s F12 supplemented with 0,1% BSA) and diluted to a concentration of 3×10⁵ cells/ml. Coelenterazine was added at a concentration of 2.5 µM and the cells were incubated on a rotating wheel at room temperature for 3 hours while protected from light. Digitonin was used as a positive control. Fentanyl, morphine and buprenorphine were used as references for full- and partial µ-opioid receptor agonists. Dose-response curves were established for all compound, on at least three separate occasions, using a 8-fold serial dilution in triplicates starting from 20000 ng/mL (~60 µM) and ending at 0.0095 ng/mL (~30 pM) in 96-well plates (100 µL/well). After dispensing 50 µL of cells into each well (15000 cells/well) the luminescence was measured for 25 seconds using a TECAN Spark 10M. The activity was expressed as a percentage of the positive control (digitonin) activity and visualized in a dose-response curve. Each 96-well plate had its own controls and each experiment was repeated three times (N=9). EC50 values with 95 % confidence interval (CI) and curve fittings were calculated using GraphPad Prism version 6.01.

Results

Full dose-response curves were obtained for cyclopropyl-, cyclobutyl-, cyclopentyl-, cyclohexyl-, 2,2,3,3-tetramethylcyclopropylfentanyl, fentanyl, morphine and buprenorphine. Cylopropylfentanyl was the most potent analog (EC50 = 2.21 nM, CI = 1.579-3.08 nM) comparable to fentanyl (EC50 = 1.96 nM, CI = 1.30-2.95 nM). Cyclobutylfentanyl (EC50 = 6.75 nM, CI = 4.45-10.24 nM) and cyclopropylfentanyl (EC50 = 6.71 nM, CI = 3.53-12.76 nM) were less potent than fentanyl but more potent than morphine (EC50 = 414.50 nM, CI = 305.00-563.40 nM). Both cyclohexyl- and 2,2,3,3-tetramethylcyclopropylfentanyl were partial agonists, reaching an efficacy at 39 % and 41 % of the full agonist fentanyl. The EC50 values were 24.19 µM (CI = 19.30-30.33 µM) for cyclohexylfentanyl and 1.48 µM (CI = 1.13-1.94 µM) for 2,2,3,3-tetramethylcyclopropylfentanyl. They were both less potent than buprenorphine (EC50 = 0.97 µM, CI = 0.98-0.45 µM).

Discussion

The main findings from the study was that even with large changes to the amide structure, the µ-opioid receptor activity was maintained but that there were considerable variations in drug potencies.

Increasing the size of the amide either by increasing the ring size or adding methyl-groups produced partial agonists and lowered the potency.

Conclusions

The µ-opioid receptor activation potencies of alicyclic fentanyls’ vary depending on their chemical structure. We conclude that the smaller the alicyclic ring, the more potent is the drug and the risk of accidental fatal intoxications caused by respiratory depression.
Abstract ID 358
Detection and phase I metabolism of the 7-azaindole derived synthetic cannabinoid 5F-AB-P7AICA including a preliminary pharmacokinetic evaluation.

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Aim
Introduction: Since 2015, several 7-azaindole derived synthetic cannabinoids (SCs) were reported. In June 2018, a ‘research chemical’ labelled as “AB-FUB7AICA” was purchased online and analytically identified as 5F-AB-P7AICA (N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-1H-pyrrrolo[2,3-b]pyridine-3-carboxamide), the 7-azaindole analogue of 5F-AB-PINACA. Here we present data on the structural characterization, the CB1-receptor binding affinity, suitable urinary markers and preliminary pharmacokinetic data of this compound.

Methods
Methods: Structure elucidation and characterization was performed by nuclear magnetic resonance (NMR) spectroscopy and gas chromatography–mass spectrometry (GC-MS) in the frame of the EU funded ADEBAR project (IZ25-5793-2016-27). Competitive radioligand binding assays were applied using [3H]-CP55,940 to measure the CB1-receptor binding affinity. As no reference standards for the metabolites were available, phase I metabolites were generated by pooled human liver microsomes (pHLM) to confirm the findings from authentic urine samples collected after oral self-administration of 2.5 mg of the parent compound. Liquid chromatography time-of-flight mass spectrometry (LC-qToF-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) were applied for this purpose. An LC-MS/MS method for the quantification of 5F-AB-P7AICA in serum was validated, and allowed to build a time-concentration profile in serum with the samples collected after oral intake of the drug.

Results
Discussion
Results and Discussion: 10 phase I metabolites were detected by LC-qToF-MS analysis of the authentic human urine specimens and all were confirmed in-vitro, following glucuronidase treatment. The main in-vivo metabolites were built by hydroxylation, terminal amide hydrolysis and hydrolytic defluorination, though the most intense peaks were seen for the parent compound in all the urine samples collected. Good linearity was achieved in the validation study in the concentration range 0.1-30 ng/mL, with a correlation coefficient (R2) of 0.9985 in the absence of a weighing factos; accuracy and precision were determined at two different concentrations, in the range of 0.8-2.22% (bias) and 3.0-4.20% (RSD), respectively. Peak serum concentration (29.5 ng/mL) of the parent compound was reached approximately 3 hours after the intake. The substance was still above the limit of quantification (0.036 ng/mL) more than 45 hours after the intake. 5F-AB-P7AICA and its main metabolites were still above the threshold of an MS peak area of 1×104 cps in urine samples 3 days after the consumption. The binding affinity of 5F-AB-P7AICA at CB1 was measured with a Kᵢ value of 3.34 ± 0.11 nM.

Conclusions
Conclusion: The present study is a further example of wrong labelling of SCs sold as ‘research chemicals’. The questionable identity of substances purchased on the Internet could lead to serious adverse events, particularly if drug potencies differ. SF-AB-P7AICA is subject to extensive metabolism in humans and only partially fits the expected pattern of urinary metabolites, as based on the profiles of other pentylinidole/indazole based synthetic cannabinoids and their 5-fluoro analogues, due to the high intensity of the parent compound. Multiple compounds, including the parent, should be included to confirm the uptake in routine urine screening, although a slightly different pharmacokinetic and metabolic pattern could arise from parenteral uptake.
Abstract ID 374

Prevalence estimation of synthetic cannabinoid use in prisons and forensic psychiatric hospitals before and after the introduction of the NpSG.

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Aim
In November 2016, the German legislative passed a new law to control new psychoactive substances (NPS). In contrast to the German Narcotics Law (BtMG) which defines single substances as narcotics, the so-called NpSG defines two classes of NPS by chemical structure: 2-phenethylamine and synthetic cannabinoids. Synthetic cannabinoids (SCs) represent the group of NPS with the highest prevalence in Germany. In prisons and forensic psychiatric hospitals SCs are often used by inmates due to the inability of immunochemical tests to reliably detect the use of these drugs. The aim of the study was to evaluate the impact of the law on the rate of NPS detection in prisons and forensic psychiatric hospitals. For this purpose, we compared a two-year time period before and after the law entered into force.

Methods
Urine samples were sent by prisons and forensic psychiatric hospitals for SC analysis in frame of routine control of abstinence. The analysis comprised liquid-liquid extraction and LC-MS/MS detection of SC metabolites.

Results
In prisons the positive rate of SCs was 48% (407 of n = 927) in the two-year period before the NpSG came into force. In the following two years the positive rate decreased to 30% (548 of n = 1,877). The positive rate of SCs in forensic psychiatric hospitals was 18% (1090 of n = 6,114) for the time period 2015 to 2016 and decreased to 13% (464 of n = 3,625) after the NpSG came into force. In particular, the SC positive rates were lower in 2017 than in 2016.

Before the NpSG entered into force, the relative proportion of SCs consumed in prisons covered by the German Narcotics Law (BtMG) was 44.5% and in forensic psychiatric hospitals 48% (2015-2016). In the following year, 70% of the detected SCs were covered by the BtMG and 10% by the NpSG in prisons. In forensic psychiatric hospitals 62% were regulated by the BtMG and 17% by the NpSG.

Frequently consumed SCs in prisons and forensic psychiatric hospitals were 5F-ADB, ADB-PINACA, cumyl-PEGACLONE and MDMB-CHMICA.

Discussion
The positive rates in prisons were generally higher than the rates in forensic psychiatric hospitals, which can probably not be translated into a higher prevalence in prisons but is rather an effect of the higher test frequency without particular suspicion in the hospital setting. The decrease of positive rates from 2016 to 2017 might be interpreted as an effect of the NpSG (supply reduction) but other explanations like increased awareness (and consequently higher sampling frequency, particularly in prisons) seems possible. However, in 2018 the positive rates seemed to increase again.

In 2017 about 80% of the consumed SCs were covered by one of the German laws in prisons as well as in forensic psychiatric hospitals. In 2018, the ratio in prisons remained largely the same. In forensic psychiatric hospitals the situation shifted to a lower BtM proportion of 48% and a negligible amount of NPS of 3%. Therefore, only about 50% of the SCs were covered by laws in forensic psychiatric hospitals for this period. In 2018, the proportion of legal substances in forensic psychiatric hospitals was comparably high as in 2016, before the NpSG came into force.

Cumyl-PEGACLONE has been subjected to the German Narcotics Law (BtMG) in July 2018 and since then, its prevalence sharply declined. The fluorinated analog of cumyl-PEGACLONE, SF-cumyl-PEGACLONE, which is still not subject to any law in Germany, was first detected in April 2018 in prisons and in May 2018 in forensic psychiatric hospitals.

Conclusions
After the NpSG came into force at the end of 2016, the proportion of ‘legal’ substances detected in prisons and in forensic psychiatric hospitals decreased to about 20%. The still relatively high percentage of ‘legal’ substances could be explained by the fact that the supply side quickly reacted with the introduction of new structural types, which are not covered by any German law so far. For example, the fluorinated cumyl-PEGACLONE derivative was found both in prisons and in forensic psychiatric hospitals shortly before the incorporation of cumyl-PEGACLONE into the German Narcotics Law. The increase of ‘legal’ substances to about 50% in forensic psychiatric hospitals in 2018 could be explained by a higher awareness of the inmates of legality aspects in combination with the possibility of direct access to online shops.
Abstract ID 379

Ultra-Sensitive Forensic Analysis of Cocaine and its Metabolites in Hair Samples.

Aymeric Morla

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Aim

Cocaine is one of the most commonly abused recreational drugs, with an estimated 16.5 million people or 0.35% of the worldwide population abusing the substance. Detection of its use can be performed in several biological matrices such as blood, urine, oral fluid and hair. While urine and oral fluid are very useful for determining cocaine use in short term, hair samples are becoming extremely valuable in testing the long-term use.

In this technical note, a sensitive and reliable analytical workflow is presented which combines the use of mass spectrometry (MS) and Solid Phase Extraction (SPE). This method for quantification of cocaine and its metabolites is shown to provide the ability to maximize selectivity when confirming and quantifying low level metabolites in hair.

Methods

The panel of analytes used in this study included cocaine and ten of its metabolites (ecgonine, ecgonine methyl ester, benzoylecgonine, norcocaine, p-OH-benzoylecgonine, m-OH-benzoylecgonine, cocaethylene, m-OH-cocaine, o-OH-cocaine and p-OH-cocaine. Cocaine-d3 was used as the internal standard. Hair samples were washed accorded to accepted laboratory procedure, dried out, cut into segments and incubated overnight at 45˚C with a 35:1 water:methanol mixture for complete digestion. The digested hair samples were cooled to room temperature, prior to being spiked with a stock standard solution mixture and extracted for LC/MS screening using a solid phase extraction procedure. Prior to analysis, the hair samples were reconstituted in mobile phase for analysis.

Analytes were chromatographically separated at 30˚C using a Phenomenex Kinetex Biphenyl column (100 x 3 mm, 2.6 µm) column. Mobile phases were formic acid in water and methanol, 600 µL/min flow rate. Mass spectrometric detection was conducted on a QTRAP® 6500+ mass spectrometer operated in positive electrospray mode with multiple reaction monitoring (MRM) MS/MS method.

Results

The recovery and matrix effect were calculated using 0.005 ng/mg of each internal standard to assess the recoveries of the analytes following the SPE procedure. Analyte extraction recoveries were demonstrated to be greater than 80%, enabling the analytical workflow to obtain sub pg/mg lower limits of quantification (LLOQ) in hair matrix for the two hydrococaine isomers. The calculated values showed that the extraction procedures yielded excellent recoveries of the analytes of interest.

Discussion

Following the SPE procedure, 10 µL of the reconstituted solution were injected for each compound. Calibration curves were generated for each of the compounds to determine limits of quantitation (LOQ). The results demonstrated excellent linearity of the generated regression curves covering linear dynamic range from 3 to 4 orders of magnitude; coefficients of variations (CVs) within 10% and good accuracies. Signal-to-noise ratios (S/N) at LLOQ were found to vary from 10 to 50. The workflow showed excellent accuracy (>95%) and precision (<15%), with excellent linearity resulting in R2 values of 0.9990 for all analytes.

Conclusions

The QTRAP® MS has the ability to easily switch between quantitative MRM scans and qualitative trap scans. Using this method, the QTRAP MS was used to acquire full scan MS/MS spectra containing the complete molecular fingerprint of cocaine and its metabolites. These MS/MS spectra can be searched against relevant library for confirmation of detection. Using this strategy, simultaneous identification and confirmation of cocaine and its metabolites by acquiring full MS/MS data and using automated MS/MS library searching.
Abstract ID 381

Rapid and low-cost determination of Carbohydrate-Deficient Transferrin (CDT) based on Fluorescence Resonance Energy Transfer (FRET).

Giacomo Musile

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Aim
Since the middle ‘70s, Carbohydrate Deficient Transferrin (CDT) has become a well-established biomarker of chronic alcohol abuse. In fact, it is known that heavy consumption of alcohol (at least 50-80 g of ethanol per day) for two weeks or more, results in an increase of CDT, i.e. transferrin (Tf) glycoforms with a reduced glycosylation (asialo- and disialo-Tf). On this basis, CDT is today widely used in the diagnostics of alcohol abuses in clinical, occupational and forensic contexts. Nowadays the determination of CDT is based on different approaches, including immunnoassays, single or multi capillary electrophoresis, and ion exchange liquid chromatography. Although instrumentally sophisticated and characterized by high automation and high productivity, these techniques show important operative costs and require well equipped laboratories and specifically trained personnel. In the present work, a new, simple and low-cost, fluorescence-based analytical method, not requiring sophisticated instrumentation, is presented as a potential tool for CDT analysis. The method, based on an original highly sensitive and specific analytical approach, uses the selective interaction between transferrin and terbium (III) [Patent: WO2015/135900A1]. This technological approach, because of inherent simplicity and low-cost is particularly suitable for application in peripheral laboratories, in a clinical context, as well as in developing countries.

Methods
The analytical method includes three main steps:
• functionalization of transferrin with terbium (III): saturation of 20 μL of serum with 1 μL of a 10 mmol/L terbium (III) solution in water; addition of 480 μL of 20 mmol/L BIS-TRIS buffer, pH 5.9;
• isolation of CDT glycoforms: at pH 5.9, asialo-Tf and disialo-Tf (CDT glycoforms), unlike the other Tf-glycoforms (trisialo-Tf, tetrasialo-Tf and pentasialo-Tf) do not interact with the home-made anion exchange cartridges. On this basis, the CDT glycoforms are isolated by pouring the 500 μL diluted serum into the cartridge and letting it flow through by gravity. The drops are collected in a tube containing 20 μL NaOH 1M;
• detection with a stand-alone fluorimeter (ex λ 298 nm; em λ 550 nm): the results are expressed as absolute fluorescence arbitrary units. Alternatively, the emission radiation can be recorded by the camera of a smartphone after removal of the native protein fluorescence with a high-pass filter (460 nm) attached in front of the objective. In this case the recorded signals are processed with an open source software.

Results
The limit of detection (LOD) was calculated as the lowest amount of transferrin producing a signal with a signal-to-noise ratio ≥ 3. Under these conditions the LOD resulted 2.5 pmol/mL. The reproducibility was assessed using two serum samples with CDT concentrations of 1.5% (normal value) and 4.9% (high value), respectively [measured with HPLC-UV-Vis], which were re-analyzed for five times in the same day and for five non-consecutive days. Using the fluorescence intensity (expressed as RFU), as the analytical signal, the intra-day imprecision in the different 5 days resulted in the range between 6.6% and 14.2% and the inter-days imprecision was better than 20%.

The analytical accuracy was also assessed by comparison with an internationally accepted method for CDT analysis with HPLC-Vis method. A neat correlation was observed between the absolute CDT associated fluorescence resulting from the proposed method and the %CDT-HPLC-Vis used as reference [R2= 0.8854]. The accuracy of the method was also evaluated by using the receiver operator curve (ROC) analysis, showing an area under the curve (AUC) equal to 0.8488.

The best performances in terms of diagnostic sensitivity (78%) and specificity (91%) were achieved using at signal fluorescence intensity of 15 RFU. This intensity value was chosen as the cut-off value to identify the samples which have to undergo to the confirmation analysis by a reference method.

Discussion
The present analytical approach has been based on the application to CDT analysis of the so called FRET phenomenon induced by terbium (III) addition to transferrin. It led to a high gain in sensitivity and specificity in comparison to the traditional methods based on the absorption of the UV-Vis light. The developed procedure is based on a cut-off separation of the terbium (III) functionalized carbohydrate-deficient iso transferrins in serum by anion-exchange cartridges followed by their determination in the eluates by direct fluorescence measurements. The procedure is manual and based on a low-cost strong anion exchange home-made cartridges. The entire process requires less than 10 minutes. The method is intended for qualitative analysis and has been tested successfully in terms of precision and accuracy, also using real serum samples with known CDT concentrations.

Conclusions
Considering its main features (low-cost, ease of operation, minimum need of instrumentation) the present method looks suitable for application in screening contexts and in non-strictly regulated environments (e.g. clinical diagnosis) as well as in developing countries or remote areas.
Abstract ID 384
Quantitative Analysis of Fentanyl and Analogues in Human Whole Blood.
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Aim
Fentanyl analogues and their metabolites are a rising concern as thousands die from opioid overdose across the country. Some of these synthetic drugs have very high potency and thus only require a small amount for an accidental overdose. In addition, these high potency drugs pose a danger to public health and public safety personnel due to the possibility of skin absorption or inhalation of the drug. In order to properly identify these fentanyl analogues in biological matrices, forensic laboratories require sensitive MS systems to accurately quantitate at low concentrations, and highly specific chromatographic methods in order to separate and properly identify isomers.

A confirmatory method for fentanyl and its analogues in human blood is demonstrated. The development of an optimized chromatographic method enabled baseline resolution of 29 fentanyl analogues in a 17-minute runtime. The column used in conjunction with an optimized mobile phase composition produced the separation that was needed to correctly distinguished all isomers. Using this comprehensive workflow, confident identification of fentanyl, its analogues, and metabolites was achieved in a complex biological matrices such as human whole blood.

Methods
Control whole blood samples were spiked with a stock standard solution mixture and extracted for LC-MS screening to determine retention times. Forensic case samples and control whole blood samples were extracted by using a protein precipitation prior to being reconstituted in mobile phase for analysis. Analytes were chromatographically separated at 30°C using a Phenomenex C18 column on the SCIEX Exciion AC system. Mobile phase consisted of water, methanol, acetonitrile and modifier, 300 µL/min flow rate. Mass spectrometric detection was conducted on a QTRAP 4500 system operating in positive electrospray mode with scheduled multiple reaction monitoring (MRM) method.

Results
The development of an optimized chromatographic method enabled baseline resolution of 29 fentanyl analogues in a 17-minute runtime. Quantification of the 29 fentanyl analogues resulted in LODs of 0.1 ng/mL for most analytes while maintaining linearity and precision for all compounds across the calibration range.

Discussion
The dynamic range for quantitation of the 29 fentanyl analogues averaged ~4 orders of magnitude across the compounds monitored in this study. These results demonstrate that the combination of mass spectrometry and highly specific chromatographic methods allows accurate quantification of these new substances while offering confident drug identification at low concentration levels.

Conclusions
The QTRAP 4500 system combined with the ExionLC AC system enabled the separation of fentanyl, its analogues, and metabolites, while maintaining the sensitivity at low concentrations in complex biological matrices such as human whole blood. The Scheduled MRM algorithm in Analyst Software 1.7 and the speed of the QTRAP 4500 system produced ample acquisition points for quality data that is easily quantifiable.
Abstract ID 387
Characterization of 4-chloroethcathinone by GC-MS after 2,2,2-trichloroethyl chloroformate derivatization, LC-HRAM OrbitrapTM MS, and solid deposition GC-FTIR.

Giampietro Frison
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Aim
The market growth of new psychoactive substances (NPS) is a complex challenge that affects both health and safety of citizens worldwide. In particular, an impressive number of new amphetamine-related designer drugs, including cathinone analogs, appear for some years now on the recreational drug market. Clinical and forensic toxicology laboratories are challenged every day by the analytical aspects of the NPS phenomenon. They are required to identify NPS, sometimes very quickly and often without the availability of reference standards or analytical data from scientific literature. Here we describe the characterization, by means of several analytical techniques, some of which have not previously been applied, of the synthetic cathinone 4-chloroethcathinone [N-ethyl-2-amino-1-(4-chlorophenyl)-propan-1-one, 4-CEC], contained, together with other more frequently detected cathinones and caffeine, in a seized powder.

Methods
The analytical techniques employed include i) gas chromatography – mass spectrometry (GC-MS), without or with derivatization with 2,2,2-trichloroethyl chloroformate, in EI full-scan (m/z 40-600) conditions with a HP-5MS UI (30 m x 0.25 mm, 0.25 µm film thickness) capillary column; ii) liquid chromatography – high-resolution accurate-mass OrbitrapTM mass spectrometry (LC-HRAM OrbitrapTM MS), using a Hypersil Gold PFP analytical column (2.1 x 50 mm, 1.9 µm particle size), a scan range from m/z 50 to 800 and mass resolution of 100.000 (HCD off) or 25.000 (HCD on, 25 eV); iii) solid deposition gas chromatography – Fourier transform infrared spectroscopy (GC-FTIR), utilizing a SLB-5MS analytical column (30 m x 0.25 mm, 0.25 µm film thickness), a 700-4000 cm-1 spectrum range, with a 4 cm-1 resolution.

Results
Accurate mass measurement of the chloroethcathinone MH+ ions had a mass accuracy of 1.88 ppm. Moreover, fully superimposable experimental and calculated MH+ isotopic patterns were obtained, with relative isotopic abundance (RIA1 and RIA2) values of 0.59 and 3.09 %, respectively. The isotopic fine structures (IFS) of the M+1, M+2, M+3 isotopic peaks were completely in accordance with theoretical values. Accurate mass measurement of the characteristic MH+ collision-induced product ions obtained from LC-HRAM OrbitrapTM MS analyses were in full agreement with the expected structures and consistent with those previously reported using low-resolution mass spectrometry. The application of GC-MS after derivatization with 2,2,2-trichloroethyl chloroformate permitted to obtain highly informative EI mass spectra. Due to the introduction of three chlorine atoms into the derivatized molecule, and the original presence of one chlorine, the mass spectrum is characterized by molecular and various fragment ions with characteristic isotopic patterns. In particular, the presence of a characteristic base peak cluster at m/z 246/248/250 confirmed the presence in the molecule of the N-ethyl moiety. The application of solid deposition GC-FTIR allowed discriminating among chloroethcathinone positional isomers (i.e. 2’, 3’, or 4’ positions of the chlorine atom on the benzene ring), confirming the presence of the 4’ isomer (4-CEC).

Discussion
The application of LC-HRAM OrbitrapTM MS allowed to obtain the accurate mass measurement of the chloroethcathinone MH+ ions in accordance with the elemental formula C11H14ClNO. The experimental and calculated MH+ isotopic patterns, the RIA1 and RIA2 values, and the IFS of the M+1, M+2, M+3 isotopic peaks confirmed the assignment of the above elemental formula. The accurate mass measurement of the characteristic MH+ collision-induced product ions, the highly informative EI mass spectra obtained from GC-MS after 2,2,2-trichloroethyl chloroformate derivatization, the identification of the chlorine atom position of the cathinone analogue by solid deposition GC-FTIR, enabled the full structural characterization of 4-CEC.

Conclusions
The combination of the described analytical techniques, some of them not applied before to the best of our knowledge for the characterization of 4-CEC, allowed the characterization of the seized psychoactive substance, in spite of the lack of a reference standard.
Abstract ID 390

Differentiation of the isomers isomephedrone and mephedrone by GC-MS after 2,2,2-trichloroethyl chloroformate derivatization, LC-HRAM OrbitrapTM MS, and GC-FTIR.

Gianpietro Frison

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Aim

Synthetic cathinones and cannabinoids represent the largest groups of novel psychoactive substances (NPS) reported worldwide. A particular analytical challenge for clinical and forensic toxicology laboratories is represented by the identification of positional and structural isomers of synthetic cathinones, to be obtained often without the availability of reference standards.

Here we describe the differentiation, by means of multiple analytical techniques as recommended by theSWGDRUG, of the synthetic cathinone isomers isomephedrone [1-(methylamino)-1-(4-methylphenyl)propan-2-one] and mephedrone [2-(methylamino)-1-(4-methylphenyl)propan-1-one, 4-MMC], contained, together with 4-chloroethcathinone and caffeine, in a seized powder.

Methods

The analytical techniques employed include i) gas chromatography – mass spectrometry (GC-MS), without or with derivatization with 2,2,2-trichloroethyl chloroformate, in El full-scan (m/z 40-600) conditions with a HP-5MS UI (30 m x 0.25 mm, 0.25 µm film thickness) capillary column; ii) liquid chromatography – high-resolution accurate-mass OrbitrapTM mass spectrometry (LC-HRAM OrbitrapTM MS), using a Hypersil Gold PFP analytical column (2.1 x 50 mm, 1.9 µm particle size), a scan range from m/z 50 to 800 and mass resolution of 100,000 (HCD off) or 25,000 (HCD on, 25 eV); iii) solid deposition gas chromatography – Fourier transform infrared spectroscopy (GC-FTIR), utilizing a SLB-5MS analytical column (30 m x 0.25 mm, 0.25 µm film thickness), a 700-4000 cm-1 spectrum range, with a 4 cm-1 resolution.

Results

Accurate mass measurement of the MH+ ions had a mass accuracy of 0.17 ppm for both cathinones. Fully superimposable experimental and calculated MH+ isotopic patterns were obtained for the two substances, with relative isotopic abundance (RIA) values of 0.59 and 0.08 % for isomephedrone and mephedrone, respectively. The isotopic fine structures (IFS) of the M+1, M+2, M+3 isotopic peaks were completely in accordance with theoretical values, even if identical for both drugs. Instead, the patterns of the MH+ collision-induced product ions were completely distinguishable for the two isomers, and in full agreement with the expected structures. The application of GC-MS after derivatization with 2,2,2-trichloroethyl chloroformate permitted to obtain highly informative and characteristic EI mass spectra of the two structural isomers, in particular due to the presence of characteristic yet different base peak clusters at m/z 308/310/312 (isomephedrone) and 232/234/236 (mephedrone). Besides obtaining a very good match for isomephedrone against a literature IR spectrum, the application of solid deposition GC-FTIR easily allowed discriminating among methylmethcathinone (MMC) positional isomers (i.e. 2’, 3’; or 4’ positions of the methyl substituent on the benzene ring). It confirmed the presence of the 4’isomer (mephedrone, 4-MMC) following a highly favorable matching of the experimental IR spectrum against the three MMC spectra contained in the IR library. The mephedrone powder content was determined to be 1%, and the isomephedrone content was estimated to be less than 0.5%.

Discussion

The application of LC-HRAM OrbitrapTM MS allowed to obtain the accurate mass measurement of the two isomeric cathinones MH+ ions in accordance with the common elemental formula C11H15NO. The experimental and calculated MH+ isotopic patterns, the RIA values, and the IFS of the M+1, M+2, M+3 isotopic peaks confirmed the assignment of the above elemental formula for both substances. Differentiation of structural isomers (isomephedrone vs. mephedrone) and positional isomers (4-MMC vs. 3-MMC and 2-MMC) was obtained by a) the evaluation of the different patterns of the MH+ collision-induced product ions obtained from LC-HRAM OrbitrapTM MS analyses; b) the highly informative EI mass spectra, due to the introduction of three chlorine atoms into the derivatized molecules, obtained from GC-MS after the straightforward and relatively rapid derivatization procedure; c) the unambiguous identification of the methyl substituent position of the MMC positional isomers by solid deposition GC-FTIR. In particular, considering the isomephedrone and mephedrone low concentration values and the relatively high number of chemicals present in the seized powder under study, solid deposition GC-FTIR has proved to be of greater applicability, owing to its high specificity and sensitivity, than nuclear magnetic resonance (NMR).

Conclusions

The combination of the described analytical techniques allowed differentiating the synthetic cathinone structural isomers (isomephedrone vs. mephedrone) and positional isomers (4-MMC vs. 3-MMC and 2-MMC) contained in the seized product.
Abstract ID 393

Studies in Fentanyl Metabolism for Forensic Toxicology: Analysis of Fentanyl and Metabolites Following the Medical Administration of Fentanyl.

Michelle Wood

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Aim

Overdose deaths from opiates and synthetic opioids have risen substantially over the past several years, with the largest increases attributed to synthetic opioids such as fentanyl and its analogs [1-2]. Identification of fentanyl and fentanyl analogs in forensic toxicology can be crucial in alerting public health authorities to potential overdose risks. Data from some of our more recent post-mortem cases seems to suggest a shift away from the synthetic analogs and back to fentanyl. Most of these fentanyl positive cases also show substantial levels of norfentanyl and 4-ANPP. 4-ANPP has been proposed as a minor metabolite of fentanyl [3]; it is also thought to be an impurity resulting from the clandestine manufacture of fentanyl. Other compounds, such as β-OH fentanyl and acetyl fentanyl have been noted at low levels in many of our post-mortem cases containing high levels of fentanyl, leading to speculation that these may also be minor metabolites of fentanyl rather than separate pharmaceutical substances. The objective of this study was to apply a sensitive and validated UPLC-MS/MS method for fentanyl agents in the analysis of plasma samples from individuals administered pharmaceutical grade fentanyl, to gain further insight into human metabolism of fentanyl.

Methods

Plasma samples were obtained from subjects at Albany Medical Center that received pharmaceutical grade fentanyl before, and during, surgery for parathyroidectomy. Subjects received 50-150 µg of fentanyl upon induction of anaesthesia and may also have received further doses depending on the patient’s sympathetic response to surgical stimuli. Blood samples were drawn pre-operatively, shortly after induction, and approximately every ½ hour as surgery progressed. The study was conducted in accordance with Albany Medical Center IRB review and approval. De-identified samples were stored frozen at -20 °C until analysis.

Two hundred and fifty microliters of plasma samples were diluted 1:1 with 4% H3PO4 (containing deuterated internal standards for fentanyl and analogs) and loaded directly onto Waters Oasis PRIME MCX µElution plates. The sorbent was washed with 200 µL 2% formic acid:100 mM NH4COOH and 200 µL MeOH. Samples were eluted with 2 x 25 µL aliquots of 50:50 ACN:MeOH containing 5% strong ammonia solution (28%) and diluted with 50 µL of 97:2:1 water:ACN:formic acid. Five microliters were analysed using a Waters Xevo TQ-S micro tandem quadrupole MS. The method was validated for recovery, matrix effects, linearity, accuracy, precision, sensitivity, and carryover.

Results

UPLC-MS/MS was achieved using a Waters ACQUITY CSH C18 column (2.1 x 100 mm) in combination with multiple reaction monitoring (MRM). Two MRM transitions were monitored for each compound; target transition ion ratios (qualifier to quantifier) were established from calibrator and QC concentrations. The total analysis time was 4 min. LLOQ values ranged from 4-5 pg/mL for the compounds analysed in this study. Concentrations of fentanyl ranged from 92 pg/mL to nearly 1500 pg/mL. The median and mean values were 336 and 393 pg/mL, respectively, with >90% of values between 100 and 650 pg/mL. Concentrations showed a general decreasing trend over time consistent with the single dose administration. Norfentanyl was found in all samples that contained fentanyl, at concentrations ranging from 5 to 47 pg/mL, with mean and median values of 19 and 17 pg/mL, respectively. β-OH fentanyl was detected in half of the fentanyl/norfentanyl positive samples. Although, in many others, it appeared to be present, but was below the established lower limit of quantification. The most consistent ratio was that of norfentanyl to β-OH fentanyl, which had a mean of 2.85 ± 1.2 (S.D.). No other compounds were detected.

Discussion

Concentrations of β-OH fentanyl were low but tracked well with the production of norfentanyl and were present at approximately 1/3 of its concentration. It also appears to be produced at a similar rate to norfentanyl, supporting the theory that low levels of β-OH fentanyl seen in fentanyl overdose cases are likely the result of fentanyl metabolism and not from a unique fentanyl analog. No acetyl fentanyl was detected in this study, although given the low levels seen in many fentanyl overdose cases, it may have been below the LLOQ. No 4-ANPP was seen in any of the samples in contrast with earlier metabolism studies and in contrast to many overdose cases where it is thought to be a byproduct of illicit production. The results of this study support the hypothesis that 4-ANPP is not a major metabolite of fentanyl and its presence is likely the result of illicit production when seen in overdose cases in forensic toxicology.

Conclusions

This study furthers the understanding of fentanyl metabolism during this current opioid crisis and will help in postmortem toxicology interpretations.

References

Abstract ID 395
Evaluation of DART QDa - an Ambient Ionization Technique Coupled with a Mass Detector for Rapid Forensic Drug Screening.

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Aim
Authorities worldwide constantly struggle to ensure public safety in the fight against the traditional recreational drug substances. In addition, the last decade has seen the emergence of more than 800 new psychoactive substances (NPS). The increase in both the number and the diversity of drugs on the market presents a significant challenge for laboratories who are involved in the analysis of seized substances. Consequently, methods that can facilitate the rapid screening of drugs are of interest. DART (Direct Analysis in Real Time) is an ambient ionization technology which permits direct analysis of drug substances with minimal sample preparation and without chromatography.

The aim of this study was to assess the feasibility of using DART QDa system for rapid drug screen and to compare data with an established screening method based on high-resolution mass spectrometry (HRMS).

Methods
A DART source (IonSense®) was coupled to a Waters ACQUITY QDa mass detector. Samples (pills, powders, resin) were analysed following dilution with methanol and spotting 3 µL aliquots onto a QuickStrip sampling card, or directly using a 10-position tablet carrier. The QDa was operated in positive ionization mode and data were acquired at four cone voltages (15, 30, 50 and 70V) to generate fragmentation by in-source collision-induced dissociation (CID). Data was processed using PIMISA software together with a spectral library containing >800 substances (IonSense).

Data was compared with an established HRMS screening method i.e. UNIFITM Forensic Toxicology Screening Solution (Waters) comprising an ACQUITY UPLC I-Class in combination with a Xevo G2-XS QTof. This reference method comprised a 15 min chromatographic separation; data was acquired using MSE which facilitates collection of data at low and high collision energies thus providing accurate mass for precursor and diagnostic fragment ions. HRMS data was compared with a library comprising >1600 toxicologically-relevant substances. Identification was based on retention time (± 0.35 min of reference analyte), precursor mass and at least one fragment ion within 5 ppm of exact mass.

Results
The DART source used a needle electrode and helium gas, delivered at 300oC, to effect ionization of analytes which takes place at ambient air, in the region between the end of the source and the inlet of the mass spectrometer. Mass detection was performed using full scan m/z 60-650, at four cone voltages. Confidence in DART identification was achieved by comparing spectra (using a reverse fit algorithm) with corresponding data in a spectral library; the average match score was used and, in this study a minimum match of 70% was used to indicate a putative positive.

The limit of detection was evaluated using methanolic solutions of certified reference material and was ≤ 20 µg/mL for the majority of analytes investigated. The study assessed various samples including: reference material, a series of prescribed medications and natural supplements/herbal medications and >60 unknown samples that had been confiscated at a UK nightclub by the local police. These data are presented.

Discussion
The intact tablet analysis demonstrated good qualitative agreement with methanolic solutions. For the nightclub samples, DART analysis indicated that 40% of the seized samples contained ketamine only, 30% contained MDMA only; 17% were cocaine only. Other drugs identified were mixtures of these three drugs or MDMA/MDEA, paracetamol, sildenafil. Two samples (3.1%) were unidentified by DART. One of these was identified as 2C-B/MDMA using the HRMS screening method. Overall there was excellent agreement with the major components identified by DART; HRMS revealed presence of additional lower concentration adulterants/impurities.

Conclusions
DART QDa is a promising technique for rapid identification of drug substances; results were obtained within 2 mins and showed very good agreement with a comprehensive screening method.
Abstract ID 399
Non-target screening method for drugs in blood by combinational use of LC/Q-TOFMS and Micro Volume QuEChERS kit.

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Aim
In the suspected cases of acute intoxication involving drugs, forensic toxicologists have to identify drugs in biological specimens such as blood and urine. High resolution mass spectrometry such as Q-TOFMS is one of the most effective techniques for non-target drug screening analysis though it is necessary to combine it with appropriate extraction methods to achieve comprehensive detection of drugs in biological fluids. QuEChERS, a protein precipitation-based sample preparation method, has been generally used for extracting drugs from biological fluids in forensic toxicology. However, conventional commercially-available QuEChERS kits show somewhat low-operativity because they require relatively large sample volume (500 μL), which is really disadvantageous to real forensic cases. New commercially-available kit, namely Micro Volume QuEChERS kit, successfully reduce initial sample volume to 100 μL of whole blood. Here, we will demonstrate the non-target screening method for drugs in blood by combinational use of a newly-developed LC/Q-TOFMS and the Micro Volume QuEChERS kit. We optimized analytical conditions of data dependent acquisition (DDA) and evaluated the practicality of our method by analyzing blood samples to which 38 drugs were spiked at 0.5-100 ng/mL.

Methods
Reagents: In total, 38 drugs are used for evaluation of the method: amphetamine-type stimulants, new psychoactive substances such as synthetic cannabinoids and synthetic cathinones, narcotics, hypnotics, antidepressants, natural toxins etc. These drugs were spiked to blood at 0.5-100 ng/mL except phenobarbital (250-1000 ng/mL). Analytical conditions: The spiked blood samples were treated using micro volume QuEChERS kit (Shimadzu, Kyoto). The obtained supernatant was evaporated at 65°C under gentle nitrogen stream, and the residue was reconstituted with a 10 mM AAc stream, and the residue was reconstituted with a 10 mM AAc aqueous solution with 0.1% formic acid and methanol were used as mobile phases, and gradient elution was performed.

Results
Our previous study demonstrated that Micro Volume QuEChERS kit showed satisfactory recovery rate for ca. 100 drugs and metabolites in human blood1, where most of them were more than ca. 80%, suggesting that there is high possibility for achieving drug screening method by combinational use of Micro Volume QuEChERS kit and LC/Q-TOFMS. To detect the target drugs comprehensively, the analytical parameters of DDA were optimized as follows: BPC, 100; period of exclusion, 60 sec; and event number, 2. The optimized DDA allows detection of the drugs that show low-signal intensities even when they were co-eluted with other drugs that shows higher signal intensities, demonstrating high data acquisition ability of the method. The detection limits for each drug were as follows: 0.5 ng/mL (atropine), 1 ng/mL (aconitine, alprazolam, fentanyl, fludiazepam, flurazepam, haloperidol, MAM-2201, methamphetamine, triazolam and zolpidem), 2 ng/mL (5F-ADB, acetylfentanyl, carbamazepine, diazepam, estazolam, etizolam, JWH-018, MDA, medazepam, oxycodeone, paroxetine, prazepam and scopalamine), 5 ng/mL (mianserin and zopiclone), 10 ng/mL (amphetamine, clonazepam, imipramine, JWH-122, lorazepam, MDMA, oxazepam, α-chaconine and α-PHP), 50 ng/mL (THC), 100 ng/mL (a-solanine) and 250 ng/mL (phendobarbital). Although THC, a-solanine and phenobarbital were detected at over 10 ng/mL, most of the drugs (ca. 90%) were detected at 0.5-10 ng/mL by the non-target analysis using LC/Q-TOFMS.

Discussion
We successfully developed the non-target drug screening method by combinational use of the new LC/Q-TOFMS (LCMS-9030) and Micro Volume QuEChERS kit, which allows sensitive detection of the analytes in blood; most of the analytes were detected at 0.5-10 ng/mL, demonstrating practicality of the method. Although some drugs such as THC and a-solanine were detected at over 10 ng/mL, which is mainly due to their low recovery rates for QuEChERS, we were able to detect them at their toxic blood concentration. Currently, we have applied the method to postmortem blood samples.

Conclusions
The newly developed non-target drug screening method by combinational use of LC/Q-TOFMS and Micro Volume QuEChERS kit achieves sensitive and comprehensive detection of drugs in small volume of blood.

Abstract ID 404
Online µSPE for fully automated LC-MS screening of urine samples in forensic and clinical toxicology.

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Aim
Systematic toxicological analysis (STA) is a major part of everyday work in forensic toxicology and immunological screening offers great advantages in automation of sample preparation and reporting of results. During the last decade, LC-MS has become a key technique in STA, but in contrast to immunoassays an appropriate sample preparation is crucial for screening of body fluids. Offline liquid-liquid extraction (LLE), solid phase extraction (SPE) or protein precipitation (PP) are often laborious but mandatory steps and their integration into the analytical workflow is the missing piece towards a fully automated routine LC-MS analysis.

The aim of this project was to implement an online µSPE to an existing LC-MS method to achieve a fully automated LC-MS screening of urine samples.

Methods
A set of compounds of different compound classes covering the retention time and mass range of the method was chosen and analysis was performed using existing routine LC-MSn (Toxytyper®) and LC-QTOF-MS (TargetScreener HR) screening methods. Three different types of cartridges, UCT C18 endcapped cartridges 10 mg (C18-10), UCT C18 endcapped cartridges 30 mg (C18-30) and UCT DAU cartridges 10 mg (DAU) (ITSP Solutions, Inc, Hartwell, GA, US) were compared according to their S/N ratios at low, med, and high concentration in pooled urine. Reproducibility of the complete extraction process was tested by tenfold preparation of pooled urine and Recovery (RE) and matrix effects (ME) were evaluated using a protocol adapted from Matuszewski et al. Furthermore, ante and post mortem urine samples as well spiked urine samples were analysed by using solid phase micro extraction (µSPE) to compare the findings with our current LC-MSn screening approach. Limits of detection were determined in pooled blank urine (n = 10) fortified with different mixtures of drugs and drugs of abuse in decreasing concentrations down to 25 ng/ml.

Results
Adjusting the cleaning steps after the different extraction steps led to no detectable carry-over caused by the µSPE system. Using DAU cartridges for sample preparation led to higher S/N ratios for six analytes (nordiazepam, fentanyl, buprenorphine, morphine and morphine-glucuronide). The C18-30 cartridge showed low absolute peak areas and was excluded from further evaluation studies.

The RSD for the complete extraction process (10 fold extraction) ranged from 5.6 to 10.9% (C18-10) and 10.0 to 14.9% (DAU) at low concentrations (25 ng/ml) at the high concentration level (100 ng/ml), the DAU cartridge showed better RSD values (1.8 to 6.8%) than the C18-10 cartridge (4.3 to 11.2%).

Recovery ranged from 50 to 90% (C18-10) and 61 to 100% (DAU) at low concentrations (27.5 ng/ml), 37 to 100% (C18-10) and 63 to 100% (DAU) at medium concentrations (275 ng/ml) and 32 to 93% (C18-10) and 53 to 91% (DAU) at high concentrations (550 ng/ml). The only compounds with no acceptable reproducibility and recovery were morphine-glucuronide and ecgonine methyl ester. Preliminary evaluation of matrix effects showed comparable effects for both cartridges. Maximum ion suppression was around 50%.

LOD of compounds found most in routine cases of the last years ranged from 25 ng/ml to 100 ng/ml.

For a set of 139 compounds the identification rate of the LC-MSn screening using µSPE could be improved from 74% to 84% (c = 100 ng/ml) and from 90% to 96% (c = 500 ng/ml). The µSPE-LC-MSn screening results of 28 urine and 22 post-mortem urine samples from real cases were in good agreement with the findings from routine analysis.

Discussion
Data from the selected set of compounds used in this preliminary evaluation proved the successful implementation of the solid phase extraction step into the analytical workflow. Regarding the results from recovery and reproducibility evaluation, the C18-10 cartridge seems to have some issues retaining early eluting compounds.

Further optimization of the protocol might increase the overall performance of the DAU cartridge but direct injection of the µSPE eluate limits the choice of solvents for extraction at the moment.

LC-MSn screening of fortified blank urine using µSPE led to similar or better results than the routine sample preparation. Due to higher sensitivity of the QTOF-MS system all compounds could be detected even at low concentrations. The practical applicability of the workflow could also be confirmed in a small batch of urine samples from real casework. All LC-MSn screening results were in good agreement with the initial routine screening.

Conclusions
The chosen hardware for online µSPE could be successfully implemented in both screening workflows, LC-MSn and LC-QTOF screening, respectively, enabling a completely automated LC-MS screening of urine samples from sample preparation to evaluation of data.
Abstract ID 407
Establishment of extraction procedure using ISOLUTE PLD+ protein and phospholipid removal column for LC-MS/MS analysis of 20 psychoactive drugs in whole blood.

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Aim
Recently, the evolution of mass spectrometry has allowed multi-targeted analysis of drugs with various physicochemical properties. For the simultaneous analysis of a range of drugs in postmortem specimens, a simple and effective sample extraction method is highly desired. ISOLUTE PLD+ protein and phospholipid removal column (Biotage, Uppsala, Sweden) offers a substantial improvement in extract cleanliness compared to traditional acetonitrile (MeCN) deproteinization and QuEChERS extraction method. Therefore, ISOLUTE PLD+ is expected to be an alternative sample preparation tool for LC-MS/MS analysis of a range of drugs in biological samples. The aim of the present study was to establish an effective sample extraction method using ISOLUTE PLD+ protein and phospholipid removal column for the simultaneous analysis of 20 psychoactive drugs (antidepressants, antipsychotics, sedative-hypnotics, amphetamines) in whole blood by LC-MS/MS.

Methods
Target psychoactive drugs
In the present study, 20 psychoactive drugs (amitriptyline, amphetamine, carbamazepine, chlorpromazine, diazepam, duloxetine, estazolam, etizolam, flunitrazepam, haloperidol, methamphetamine, mianserin, nitrazepam, olanzapine, paroxetine, phenobarbital, promethazine, quazepam, risperidone, and zolpidem) were selected, which had been detected in the autopsy specimens in our laboratory.

Sample extraction
A 100 μL of whole blood was diluted (1:1 (v/v)) with 200 mmol/L NaH2PO4/Na2HPO4. After addition of internal standards (diazepam-d5 and phenobarbital-d5), 700 μL of MeCN and 200 μL of methanol (MeOH) to the diluted blood sample, the sample solution was loaded onto an ISOLUTE PLD+ column attached with a centrifuge tube. The column was centrifuged at 670 g for 10 min to collect purified analytes. The eluate was evaporated with a centrifugal evaporator. The residue was reconstituted in 100 μL of MeOH and mixed by vortexing for 1 min.

LC-MS/MS
The LC-MS/MS instruments used were a Nexera X2 and LCMS-8040 system (Shimadzu, Kyoto, Japan). For separation, a Kinetex column (2.1 mm I.D. × 100 mm, particle size 2.6 µm; Phenomenex, Cheshire, UK) was used. The column temperature was maintained at 40°C, and the gradient system was used with a mobile phase A (0.1% formic acid in 10 mmol/L ammonium formate aqueous solution) and mobile phase B (0.1% formic acid in 10 mmol/L ammonium formate MeOH solution) delivered at 0.3 mL/min. A linear gradient ramped from 5% B to 95% B in 7.5 min. 95% B was kept for 2.5 min. The mobile phase was then returned to 5% B within 0.01 min, which was kept for 5.0 min to equilibrate the column for the next sample. Quantification was made in selected reaction monitoring mode using peak areas.

Results
The ISOLUTE PLD+ method with basic condition (pH 9) provided higher recoveries of olanzapine, chlorpromazine and promethazine (85.4-97.1%) which were poorly recovered with MeCN deproteinization (without pH adjustment) and QuEChERS extraction method (35.4-42.3%). The method was advantageous for its handling simplicity owing to centrifugation procedure. The method also showed better sample clean-up effect by eliminating co-existing substances in the samples such as phospholipids, lyso-phospholipids and proteins, the main causes of ion suppressions, than MeCN deproteinization and QuEChERS extraction method.

Discussion
ISOLUTE PLD+ method could be used without blocking or plugging because good fluidity of the solution was provided by increasing the proportion of MeOH. The use of ISOLUTE PLD+ column under the basic condition achieved higher recoveries of all drugs tested. The method using centrifugation procedure improved its work efficiency and handling simplicity while ISOLUTE PLD+ column was designed using either positive pressure or vacuum based systems.

Conclusions
The ISOLUTE PLD+ method demonstrated to be a highly effective but extremely simple clean-up for LC-MS/MS analysis of 20 psychoactive drugs in whole blood. Additionally, we will present the validation data including matrix effect study in the annual meeting.
Abstract ID 409
Detection and Analysis of Fentanyl
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Aim
In recent years, researches on fentanyl drugs have been mostly applied in clinical monitoring of blood concentration, providing a basis for pharmacokinetic. The purpose of this paper is to study the limit of detection(LOD) of fentanyl in urine by using the method of alkali extraction combined with GC/MS. Explore whether derivative reagents have an impact on the LOD. We hope that it can provide reference for the development of the related fields in forensic toxicology.

Methods
Various methods have been used to detect fentanyl and its derivatives in biological samples domestic and overseas. In the study, SKF525 was used as an internal standard indicating the detection status of fentanyl. The liquid-liquid extraction method was adopted to extract fentanyl from urine. To detect the content of fentanyl in the biological samples, Gas Chromatography/Mass Spectrometry (GC/MS) was used. Fentanyl standard with concentration of 100 μg /ml was added to the blank urine in a certain amount, setting a concentration gradient from 1 μg /ml to 1 ng/ml. NAOH was used to adjust the PH of the sample to 12. 20μl SKF525 solution was added, and then 3ml diethyl ether was added, mixed for 5 minutes, and centrifuged for 5 minutes. The supernatant was dried by a nitrogen blower, and then 150μl acetonitrile was used to dissolved the analyte. The samples with variable concentration were detected by GC/MS. In order to study the effect of derivation, Bis(trimethylsilyl)trifluoroacetamide(BSTFA) was added to the samples after acetonitrile dissolution. Finally the samples were derivated at 65°C for 20 minutes for detection.

Results
The limit of detection(LOD) of fentanyl by GC/MS in urine was about 30 ng/ml by using above method. LOD had no change after derivation, indicating it had no significant effect on the detection of fentanyl.

Discussion
At present, there are many investigators working on the detection and analysis methods of fentanyl drugs in plasma, but very few studies focused on fentanyl and its derivatives in urine and hair. It is suspected that, on the one hand, the metabolic rate of fentanyl and its derivatives in hair has not been determined, on the other hand, it may be because the content of fentanyl and its metabolites in hair is too rare to detect.

In addition, biological samples are usually directly used for detection and analysis after solid phase extraction or liquid-liquid extraction, the process of derivatization is also uncommon. We hypothesize that it is because the derivation do not have a significant effect on the detection process of fentanyl drugs, and the accuracy and sensitivity are not greatly affected.

In the future, we'll focus on the detection of fentanyl in hair by animal experiments.

Conclusions
GC/MS and alkali extraction can be used to detect fentanyl in urine. The limit of detection(LOD) of fentanyl by GC/MS in urine can reach 30 ng/ml. Comparing with other pretreatment methods, alkali extraction is more effective and convenient. What’s more, derivation had no significant effect on the detection of fentanyl.
Determination of Time since Deposition (TsD) of biological crime scene traces by mass-spectrometry based proteomics.

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Aim
By comparing the individual-specific DNA profiles, a piece of evidence can be assigned to a person without any doubt. Evidence should not only be associated with a person, but also with the crime itself, e.g. by determining the type of evidence, the age of the evidence and the method of transmission. To date, there is no reliable method for determining the age of a trace. The aim of this study was to investigate time-dependent changes in bloodstains as one of many forensically relevant body fluids by analysing the proteome of a sample (global proteome profiling) and to then draw conclusions about the age of biological traces and to determine the Time since Deposition (TsD) of forensically relevant crime-scene traces. Samples should be exposed to two different environmental conditions at different time points in a longitudinal study. The work presented here focuses on initial investigations regarding blood samples aged under controlled and environmental conditions.

Methods
Blood samples were created by applying 50 µL of venous blood to Whatman 903 protein saver cards and drying them for 2 hours at ambient conditions. The dried blood spots were then left to age under two conditions: Controlled (lab drawer, room temperature, 40-60% humidity, no sunlight exposure) and environmental (outside, but sheltered from direct precipitation). Aging was stopped by punching out pieces of 3 mm in diameter and storing them at -80 °C. Proteins were extracted from fresh and stepwise aged (fresh, 1 day, 3 days, 7 days, two weeks, four weeks and 12 weeks) blood spots with ammonium bicarbonate. The samples were vortexed, sonicated and subsequently centrifuged. The supernatant was transferred into fresh vessels and the total protein content was measured. For each sample, 50 µg of protein were reduced, alkylated and proteolytically digested with trypsin. After sample clean-up using C18 micro-columns, the peptides were dried using a SpeedVac concentrator (Thermo Scientific) and stored at -20 °C in a freezer. Prior to analysis, the dried peptides were resolubilized in 3% Acetonitril and 0.1% Formic acid in water followed by sonication for 10 minutes, diluted and then analysed in randomised order with nano High-Performance Liquid Chromatography (nanoHPLC, Waters nanoAcquity M-Class, Acquity M-Class HSS T3 column, 1.8 µm, 75 µm x 150 mm) and Fourier-Transformed Tandem Mass-Spectrometry (FT-MS/MS, Thermo Scientific Orbitrap Q-Exactive HF) in data-dependent acquisition (DDA) mode. Progenesis QI for Proteomics software (Nonlinear Dynamics) was used to get quantitative profiles for individual peptides and proteins for each sample. Mascot software (Matrix Science) was used with a target-decoy human database downloaded from UniProt in combination with Scaffold v4 (Proteome Software) to evaluate and filter the identified peptides and proteins present in a blood sample.

The quantitative changes over time were evaluated with a regression model on both peptide and protein level. For this purpose, protein and peptide abundances were subsumed that showed no significant time-dependent changes and compared with those that either increased or decreased in a time-dependent manner. The resulting log-ratios were plotted for each time-point and used to create a linear regression model for TsD-estimation.

Results
Identification of a biological trace was done by searching the data for the presence of body-fluid specific proteins or peptides thereof. Contamination with other organisms could be detected by the presence of proteins and peptides of microbial origin by searching a multispecies database such as Swiss-Prot or HOMD (human oral microbiome database). Different proteins showed varying degrees of time-dependent degradation, which is caused by a decrease in measurable quantity over time for various proteins. At the peptide level, typical time-dependent changes were found in the altered abundances of post-translational modifications (PTMs), such as methionine oxidation and asparagine deamidation. Further, an increased formation of semi-tryptic peptides was observed, indicating non-tryptic amino-acid sequence breaks.

Discussion
The presented (initial) results are promising in terms of feasibility for the determination of the TsD of biological crime scene traces. The proteome of a biological sample shows complex dynamics over the course of time. Aged traces provide numerous possibilities (and challenges) to tackle the question of TsD-determination. For instance, changes in type and abundance of PTMs can be observed with increasing age of a blood stain. These changes can be used to create patterns for TsD determination of a given blood stain.

Conclusions
Our first investigations show that the analysis of the proteome at both protein and peptide level may well be suitable to visualize time-dependent changes of a body-fluid derived trace. Further investigations will be necessary to show robustness of the analytical technique as well as to improve the quality of the model used for TsD-determination. Other forensically relevant biological matrices (saliva, semen, vaginal secretion and menstrual blood) need to be investigated as well.
Abstract ID 411
Recommendations for the determination of matrix suppression in biological samples by UPLC-ESI-MS/MS: Extending Quality Measures in Forensic Toxicology.
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Aim
In quantitative analysis utilizing High Performance Liquid Chromatography (HPLC)-Electrospray Ionization-Mass Spectrometry (ESI-MS), ion suppression or enhancement of analytes due to incomplete removal of matrix components can negatively impact analytical methods. These effects include altering the signal intensity of target analytes and decreasing quantitative accuracy. Based on the Academy Standards Board’s Method Validation Guidelines in Forensic Toxicology, suppression/enhancement can be evaluated using different approaches including post-column infusion or post-extraction addition. By using one of these two approaches, laboratories can assess extraction efficacy to gauge impact on crucial validation parameters. The evaluation of matrix effects is a common part of the method development process for quantitative LC-MS. The objective of this study was to demonstrate the use of post-column infusion as a tool to evaluate the effectiveness of different sample preparation methods to reduce such matrix effects.

Methods
De-identified, drug-free pooled human blood (Equitech Enterprises, Kerrville, TX, USA) (n=8) was extracted at Boston University School of Medicine. Extracts were shipped to PerkinElmer (Shelton, CT) for analysis. The analytes morphine, benzoylecgonine, 11-nor-9-carboxy-D9-tetrahydrocannabinol, fentanyl, 6-monoacetylmorphine, and hydrocodone (Cerilliant Corporation, Round Rock, TX, USA) were selected to capture the effect of matrix suppression on analytes that elute at different time points during a chromatographic run. Different sample preparation techniques were compared. Protein precipitation, phospholipid depletion, and solid phase extraction were evaluated separately and in combination. Further comparison of human blood from six different sources was further evaluated. The instrument used was a PerkinElmer QSight™ 220 MS/MS coupled to a PerkinElmer UHPLC (Shelton, CT, USA).

Results
It was suspected that early eluting analytes would be most affected by salts. It was determined that later eluting analytes were most affected by phospholipids via MRM monitoring of known lipid and phospholipid transitions. Protein precipitation and phospholipid depletion alone were not sufficient at removing suppression effects in the later part of the chromatographic run, which would affect later eluting analytes, in this case THC-COOH. Sample preparation including SPE were most successful at removing interferences. SPE of pooled blood compared to the solvent blank showed minor differences. SPE with PLD and/or PPT were nearly identical to the solvent blank (not shown). However, due to additional cost and analysis time, SPE was chosen to further evaluate its ability to minimize suppression effects across the chromatographic run. SPE was performed on human blood from six different sources to evaluate potential variation from sample to sample.

Discussion
The post-column infusion approach was effective in mapping the suppression events versus retention time to help predict future issues with quantitation for these specific analytes. PPT and PLD both showed significant ion suppression after 5.8 minutes in the chromatographic run. Combinations including SPE were the most successful, with SPE followed by an orthogonal clean up step (PPT and/or PLD) being the best, near identical to the solvent blank. However, pairing these with SPE would be more expensive and time-consuming compared to a traditional SPE method. SPE was successful at minimizing matrix effects, such as phospholipids, across the chromatographic run. Analysis of pooled blood vs. the six individual samples showed little variation.

Conclusions
Overall, the use of post-column infusion was effective at mapping matrix suppression and enhancement events. The approach provided more information on the potential negative matrix effects for each type of sample preparation approach evaluated.
Abstract ID 412
Metabolism of isobutyrylfentanyl modelled by human hepatocytes and analyzed by LC-QTOF-MS.
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Aim
Despite the appearance of new fentanyl analogs in the recent years, little is known about their metabolism. This study aimed at characterizing the metabolic pattern of isobutyrylfentanyl with the intent of comparing it to that of the structurally similar analog 4-fluoro-isobutyrylfentanyl and determining possible biomarkers.

Methods
Isobutyrylfentanyl reference standard was diluted in Williams E buffer to a final concentration of 5 μM and subsequently incubated with primary human hepatocytes (1 million cells/mL, BIOIVT 10-donor pool, cryogenically stored). Incubations at four time-points (0, 1, 3 and 5h) were performed in duplicates along with a degradation control, negative control and a positive control. The extracts after acetonitrile precipitation were analyzed on an Agilent 1290 UHPLC – 6550 QTOF-MS system equipped with an Acquity HSS T3 column (150 × 2.1 mm, 1.8 µm) and a JetStream interface with positive electrospray ionization. The aqueous and organic mobile phase consisted of 0.05% formic acid in either 10 mM ammonium formate or acetonitrile respectively and were set to run at an increasing gradient of organic solvent from 1% and reaching 40% at 13 minutes followed by a final washing by ramping up to 95%

MS-, and MS/MS data analysis was performed using Agilent MassHunter Qualitative Analysis B.07.00. The search parameter was set to allow mass errors of 5 ppm (exceptions were made for saturated peaks). All included possible metabolites displayed at least one interpretable product ion spectrum and was present in more than one sample with a minimum intensity of 20,000 (all replicates considered as individual samples).

Results
In total, thirteen metabolites were identified, all eluting within 8.56 to 13.51 min with the parent eluting at 12.27 min. Metabolites were generated either by N-dealkylation, hydroxylation, dihydrodiol formation, methylation, amide hydrolysis or combinations thereof. Norisobutyrylfentanyl (M1) was the most abundant metabolite accounting for 59% of the total metabolite peak area. The second largest peak area (26%) among the metabolites belonged to a monohydroxy metabolite (M11) which most likely was hydroxylated at the beta-carbon on the ethyl linker. The third most abundant metabolite (M13) accounted for roughly 7% of the total peak area and was another monohydroxylated metabolite. M13 eluted 1.25 min after the parent, suggesting the hydroxy group was located at the nitrogen of the piperidine ring, i.e., an N-oxide.

The M1, M11, and M13 metabolites accounted for the majority of the total peak area and most other metabolites included various mono-, and dihydroxylated metabolites as well as two dihydrodiol metabolites, one methylated dihydroxy metabolite, and N-Phenyl-1-(2-phenylethyl)-4-piperidinamine (4-ANPP). Apart from M13, the only metabolite that eluted after the parent was a dihydroxy metabolite (M12).

Discussion
These results aligned quite well with those previously reported for the structurally related 4-fluoro-isobutyrylfentanyl. In both sets of data, the nor-metabolite was the most abundant metabolite. Both analogs generated a monohydroxy metabolite that showed similar fragmentation patterns. Comparing these fragmentation patterns with those of synthesized references led us to conclude that the hydroxylation had taken place at the β-carbon on the ethyl linker. Similarly, both substances yielded a monohydroxy metabolite eluting after the parent. This is consistent with hydroxylation on the piperidine-nitrogen yielding an N-oxide metabolite. Likewise, the dihydroxy metabolite (M12) eluting at 12.77 minutes, 0.5 minutes after the parent, probably contains an N-oxide. The presence of an N-oxide among the major metabolites is quite unusual. However, the late retention time and fragmentation pattern support its existence. Though the product ion spectrum of M12 itself does not contain any fragments that include the hydroxy group, the M12 dihydroxy metabolite does. The similar fragmentations along with the slightly reduced retention time of M12 seem to indicate that M12 is formed by a combination of the M13 metabolite (N-oxide) and M11 metabolite (hydroxylation at the β-carbon on the ethyl linker).

As the three most abundant metabolites account for approximately 92% of the total peak area, they are proposed as suitable biomarkers. However, norisobutyrylfentanyl (M1) does not retain the whole structure of the parent compound, and therefore, should not be deemed to be a conclusive evidence of isobutyrylfentanyl consumption. Also, it is important to keep in mind that the relative abundance of different metabolites can differ considerably between hepatocytes and authentic urine samples. To identify the major metabolites in urine, a number of authentic isobutyrylfentanyl positive urine samples need to be analyzed.

Conclusions
The metabolism of isobutyrylfentanyl was similar to what has been reported for 4-fluoro-isobutyrylfentanyl. Norisobutyrylfentanyl (M1), the beta-position monohydroxy metabolite (M11) and the N-oxide metabolite (M13) were the major metabolites of isobutyrylfentanyl and might, together with the parent, be suitable biomarkers of isobutyrylfentanyl intake. However, further analysis of urine samples and synthesized reference compounds are necessary to confirm these results firmly.
Abstract ID 413
Synthesis, determination of metabolites and in vitro cytotoxicity of deschloroketamine enantiomers.

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Aim
Dissociative anaesthetics, the N-methyl-D-aspartate (NMDA) antagonists, that are known for their anaesthetic properties, are mainly represented by a group of arylcyclohexylamines (e.g. PCP, ketamine, methoxetamine and deschloroketamine). Dissociative anaesthetics belongs to the group of New psychoactive substances (NPS), a global phenomenon, spread as a grey market response on drug control policy. New psychoactive substances (NPS) as unregulated drugs are trying to circumvent legislative regulations by altering the structure of illicit drugs. Deschloroketamine (DXE) is often mentioned as a replacement for ketamine and methoxetamine, thus the pharmacological properties are part of the complex study of the substance. In this study, we aim to determine differences in cytotoxicity of racemic deschloroketamine and its enantiomers. We also focus on metabolomics study in urine samples and quantification of metabolites in male Wistar rats tissues, which were gained within the behavioural study on DXE held in the NIMH of the Czech Republic.

Methods
Metabolites of DXE were designed according to previous reports on ketamine and methoxetamine. These first stage suggestions were compared with LC-HRMS analysis of male Wistar rats urine after 30 mg/kg s.c. DXE administration (3 replicates). We suggested a convenient preparation method which was used for the synthesis of deschloroketamine, four deschloroketamine metabolites and a deuterium labelled derivative as analytical standards. The multistep synthesis started from bromobenzene and was based on Grignard reaction, regioselective opening of the epoxide and catalytic hydrogenation. The synthesis of deuterium labelled metabolite dihydroDXE-d4 started from norDXE. The synthesized racemate was resolved by preparative scale HPLC performed on a chiral column with tris[3,5-dimethylphenylcarbamoyl]amylose selector. The absolute configuration of the enantiomers was assigned using a combination of circular dichroism methods and single-crystal X-ray. Quantification of DXE and its metabolites in rat tissues by LC-MS/MS has been done and collected data were used for the pharmacokinetics. The in vitro cytotoxic profile of deschloroketamine and its two enantiomers was assessed using cell metabolic activity assay WST-1 after 72 h of the compound’s treatment. The IC50 values were determined as the concentration necessary to kill 50% of cells. Live-cell fluorescence microscopy imaging of SH-SYSY cells were performed after 72 h treatment with deschloroketamine (rac-DCK) and its enantiomers ((S)-DCK and (R)-DCK) at 500 mM concentration.

Results
DXE, dihydrodeschloroketamine, dihydrondeschloroketamine, and nordeschloroketamine and other six metabolites were identified in male Wistar rat urine owing to LC-HRMS. Enantioseparation achieved high efficiency to produce enantiomers in high 99% purity. We found that (S)-deschloroketamine to compare the (R)-deschloroketamine exhibited higher cytotoxicity in the majority of cases. For human embryonic kidney cells (HEK 293T), the (S)-enantiomer reached the IC50 below 1 mM concentration. Live-cell fluorescence microscopy imaging at sub-IC50 concentrations provided evidence for only a minor effect of deschloroketamine racemate and enantiomers on endoplasmic reticulum stress and mitochondria morphology in neuroblastoma cells SH-SYSY. However, at 500 µM concentration rac-DCK as well as (S)-DCK and (R)-DCK treatment resulted in ER stress pronounced by aggregation of the ER network.

Discussion
The comparison of our found metabolites and previously reported ketamine and methoxetamine metabolites suggested the same metabolic pathways. (S)-deschloroketamine exhibits higher cytotoxicity than the (R)-enantiomer, which is in line with published data of ketamine enantiomers. However, the observed toxicity is most probably not induced via a stereosensitive receptor. Previously was described significant impact of ketamine on mitochondrial function at 20 µM concentration after 24 h treatment. To compare, we have found that 250 µM concentration (72 h), neither (rac)-DCK nor its enantiomers had impact on the morphology of mitochondria and endoplasmic reticulum. In addition, it seems to harm the urinary track to a lower extent than ketamine, which may also be beneficial for potential medical application.

Conclusions
We have synthesized and characterized rac-deschloroketamine and its most abundant metabolites. Enantioseparation achieved high efficiency to harvest the pure enantiomers, (R)- and (S)-deschloroketamine. The absolute configuration of the single enantiomers has been determined and confirmed by single-crystal X-ray. The toxicity of deschloroketamine can be considered similar to ketamine and R-enantiomer can be more suitable for potential use in medicine, since it is less toxic than the S-enantiomer. These results are highly promising, since deschloroketamine is more potent and its effects are longer lasting than those of ketamine. Such properties may be highly beneficial for the potential medical use of deschloroketamine, therefore, its antidepressant effect should be thoroughly investigated.

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Abstract ID 416
Paper-based detection of 25-NBOMe compounds in oral fluid.
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Aim
Paper based systems and in particular, microfluidic devices (µPADs) have been studied for a range of analytes. Typically the focus has been on explosives detection in a forensic sense, however, more recently drug analysis has been on the rise. Commonly the detection methods are colourimetric due to the simplicity and compatibility with low cost reporting systems (e.g. smartphones, scanners). The use of colourimetric detection methods on white paper is advantageous as it provides a clear background for the colour to be viewed. This study aimed to apply a previously developed colour spot test method to paper based chromatographic systems and to also expand the analysis to detect 25-NBOMe compounds in oral fluid samples.

Methods
The adaptation of the developed colour test method to a paper based system required several considerations. The sequence of addition of the reagents and the drug solution was important in the final method along with volumes and ratios of these reagents. Analysis of the drug as a pure sample was completed first to assess the compatibility of the reagents on paper followed by optimisation of the method for detection in oral fluid samples. A simple paper chromatographic method was chosen to analyse the samples utilising a chloroform and ethyl acetate (50:50) mobile phase. Oral fluid samples (0.5 mL) were spiked with 25E-NBOMe (100 µL, 1 mg/mL) and spotted onto a Whatman No. 1 filter paper strip (6 x 3 cm) alongside an oral fluid blank. The paper strip was added to the solvent solution and removed once the solvent reached approximately 1 cm from the top of the strip. The system was then developed with a combined solution of the colour test reagents via capillary action. The methods were tested for repeatability and reproducibility, limits of detection were determined through concentration studies and potential impurities and other drugs were analysed to assess the selectivity of the test in this matrix.

Results
Several paper based systems were tested throughout this study. Initially the detection of 25-NBOMe compounds was successful, showing the expected blue colour change, on a paper substrate when the reagent was added to the paper prior to application of the drug in the buffer solution. In comparison, no colour change was seen with the other illicit drugs that were also analysed. Direct addition of the colour test reagents to oral fluid sample did not provide a positive result. Oral fluid samples which were analysed using the paper chromatographic system, first with the chloroform and ethyl acetate followed by addition of reagents afforded a positive blue colour change within minutes. The colour appeared separate, following the solvent, from the main components of the oral fluid which remained at the origin and which were visible under UV light. As a comparative tool, the reagents were also applied through capillary action without the sample strip having the chloroform and ethyl acetate step applied. This showed a less promising results and a slight blue colour change was seen at the origin, not having been separated from the oral fluid. Method validation analysis proved a reasonable level of precision through repeatability and reproducibility studies. The limit of detection was determined to be 0.2 mg/mL and no other drugs or impurities that were tested produced a colour change.

Discussion
The application of the method to simple drug sample was simplistic and could provide potential device possibilities for on-site testing. Similarly, to the previously developed colour test, the test showed high levels of precision and selectivity along with reasonable LOD values. The application of this to oral fluid samples required many methods to be trialed before a colour change was seen. A simple chromatographic method was successfully applied, and the drug could be detected at low levels. Further developments could include reduction of solvent usage and potentially substituting the hazardous chloroform for another solvent. The time required for this method to be completed may be further explored to make this a viable on-site testing method. Currently, the method takes 10-15 minutes to complete with a colour change appearing almost instantly after the final reagents are added. While this study only completed testing on oral fluid samples, other biological matrices or even complex seized drug samples may also have the potential to be analysed using a similar method. This type of analysis also has the potential to be combined with light to provide a more discriminative result. Both white and UV light may be used to accurately identify the colour change occurring and indicate with confidence the drug that is present.

Conclusions
A simple paper-based system for the detection of 25-NBOMe compounds as pure samples and in oral fluid samples has been developed in early stages. With further development and validation this could potentially become an ideal on-site testing device for these compounds.
Abstract ID 435
Comparison of internal standards for determining glyphosate, glufosinate and their metabolites in human Plasma by LC-MS/MS.
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Aim
Glyphosate (GLYP) and glufosinate (GLUF) are herbicides used worldwide. Their fatal intoxication cases often occur whereas fatal intoxication cases due to paraquat and organophosphorus pesticides have decreased during previous decades in Japan. GLYP, GLUF and their metabolites have high polarity and non-volatility; it is thus difficult to analyze them especially in biological specimens. The structures of the GLYP, GLUF and the metabolites are different to each other and therefore, all of the compounds cannot be precisely quantified using the same internal standard (IS). We compared several IS compounds to optimize the determination of GLYP and GLUF and their metabolites, aminomethylphosphonic acid (AMPA) and 3-methylphosphonicopropionic acid (MPPA) in human plasma samples.

Methods
LC–MS/MS analyses were performed on an ACQUITY UPLC System connected with an ACQUITY TQD System. For LC separation, a Scherzo SS-C18 separation column (150 mm x 2 mm i.d., particle size 3 μm) was used. A gradient elution system was used with mobile phase A (10 mM ammonium formate) and mobile phase B (200 mM ammonium formate/acetonitrile 50/50) at a total flow rate of 0.5 ml/min. The gradient program was started at 95 % mobile phase A and 5 % mobile phase B, was changed linearly to 75 % A and 25 % B for 30 sec, and was then kept at 75 % A and 25 % B for 4.5 min. For deproteinization, the mixture of a 200 μL of 100 times diluted plasma sample containing GLYP, GLUF, AMPA, MPPA, GLYP13C215N (IS), (±)-2-amino-4-phosphobutylic Acid [(±)-AP-4, IS] and DL-2-amino-5-phosphopentatonic acid (AP-5, IS) and 800 μL of acetonitrile was centrifuged at 15,000 rpm for 10 min, and the supernatant was dried up. The purified compounds were derivatized with 200 μL of acetate/acetic anhydride (98/2) and 200 μL of trimethyl orthoacetate at 120 °C for 60 min. The dried residue was reconstructed with 10 μL of acetonitrile and 90 μL of 10 mM ammonium formate. Ten microliters of the aliquot was subjected to the UPLC-MS-MS system. The m/z values of precursor and product ions for GLYP, GLUF, AMPA, MPPA, GLYP13C215N, AP-4 and AP-5 were 254 and 102, 252 and 210, 182 and 111, 181 and 149, 257 and 105, 268 and 208, 282 and 222, respectively. For quantitation, the area of each product ion peaks was measured in the selected reaction monitoring (SRM) mode.

Results
All the compounds were eluted within 3 min and sufficiently separated. The recoveries were above 36 % for the compounds except AMPA. Calibration curves showed satisfactory linearity in the range of 200 ng/mL and 10 μg/mL for each compound, using all of the three ISs. The accuracies and precisions were assessed by determining plasma specimens spiked with GLYP, GLUF, MPPA and AMPA at 200 ng/mL, 1 μg/mL and 10 μg/mL. As an IS, GLYP13C215N, AP-5 and (±)-AP-4 were the most suitable IS for GLYP, GLUF and AMPA, respectively. Although GLYP13C215N seemed to be the most suitable IS for MPPA, the accuracy was 54 % at 200 ng/mL. Except MPPA, the accuracies were in the range of 94 and 118 %, and the precisions in the range of 5.3 and 17%.

Discussion
In previous reports, DL-2-amino-3-phosphonopropionic acid (APPA) was used as IS; our previous study showed that APPA was not suitable as IS for determining GLYP and other compounds. The use of GLYP13C215N, (±)-AP-4 and AP-5 has enabled GLYP, GLUF and AMPA to be determined more precisely. For determining MPPA, another compound need to be tested. One aspect to consider would be its structural similarity.

Conclusions
The present method has enabled GLYP, GLUF and AMPA to be determined in a small amount of plasma samples. The analysis of the specimens of a fatal GLYP intoxication case will be provided.
Abstract ID 439
The role of formaldehyde in the mechanisms of acute methanol intoxication.

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Aim
Methanol is a low-molecular weight alcohol far more toxic than ethanol. As little as 10-15 mL of pure methanol can cause permanent blindness by neuronal damage of the optic nerve and 30 mL may be potentially fatal if treatment is delayed. Methanol is metabolized to formaldehyde in a process mediated by alcohol dehydrogenase, mostly in the liver. Formaldehyde is converted to formic acid by aldehyde dehydrogenase. It is well known that formate is toxic because it inhibits mitochondrial cytochrome c oxidase, causing hypoxia at the cellular level, and metabolic acidosis, among a variety of other metabolic disturbances.

However, the mechanisms of methanol toxicity may be related to the first metabolite of methanol, highly reactive formaldehyde, as well. We have performed a series of model experiments demonstrating validity of the concept.

Methods
As model compounds we have used various amino acids and medium- and large-size peptides. Cysteine, lysine, and glycaminide dissolved in water have been reacted with formaldehyde at 37 °C and the products of the reaction have been tracked by low and high resolution mass spectrometry (MS) utilizing electrospray ionization (ESI) in combination with direct infusion into the mass spectrometers. Similarly, medium and large peptides, specifically, VG30 (VGSSYLAWYQQKPGQAPRLLYGAFSRATG; Mw=3286), SY30 (SSYTMHWVRQAPGKGLEWVTIFSYDGNKY; Mw=3536), and parathyroid hormone human (Mw=9425) have been let to react with formaldehyde and then the product analysis has been accomplished via several techniques including matrix assisted laser desorption/ionization (MALDI), direct infusion into MS, and by liquid chromatography in combination with mass spectrometry (LC-MS) utilizing low and high resolution MS detection. For liquid chromatography Thermo Scientific Ultimate 3000 system has been used, low resolution MS measurement has been carried out with 3200 QTRAP (Sciex) and for high resolution MS data acquisition Q-TOF Impact II (Bruker) has been employed.

Results
In the above-mentioned experiments, in general, two types of the main reaction products have been obtained, specifically, Schiff bases (RN=CH2, + 12 Da), and hydroxymethylated compounds (RNHCH2OH, + 30 Da). However, the major reaction products were indeed Schiff bases. Similarly, for the model peptides the molecular weight of the main products corresponded to +12 Da gain in mass. Thus, again RN=CH2 imines have been formed primarily.

Discussion
Theoretically, the reaction of amino acids and peptides with highly reactive formaldehyde can provide various products including for instance a dimer formation. However, in the real experiments, the dimer formation has not been recorded. The formation of the imines seems to be a preferable route of the reaction at least in the case of amino acids and peptides studied.

Conclusions
Based on our experimental data, we conclude that the hypothesis that various amino acids could undergo chemical modification in the presence of reactive formaldehyde as the first metabolite of methanol oxidation in the human organism has been proved experimentally. It is important to note that described modifications can lead to functional changes of many key enzymes. Thus, formaldehyde may present higher risk than formic acid alone, which effects are thoroughly studied in the acute methanol intoxication context.
NPSs analysis in biological samples: from GC-MS to LC-HRMS NPSs.

Sara Odoardi

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Abstract

The aim of this study was to develop and validate rapid and efficient methods, using different hyphenated techniques, for the identification of many classes of NPS in urine, blood and hair using sample preparation techniques that maximize the time and cost but maintaining a high level of sensitivity and specificity. Instrumental methods were developed using three different analytical techniques (GC-MS, LC-MS/MS and LC-HRMS), to enable the screening of NPSs by the majority of forensic or clinical laboratories, with different instrumental availability.

Methods

Two methods were developed for urine and blood in GC/MS, one for the analysis of stimulants, using hexyl chloroformate derivatization followed by DispersiveLiquid-Liquid-MicroExtraction (DLLME), and the other one for synthetic cannabinoids, extracted by Ultrasound Assisted-DLLME (UA-DLLME), and derivatised as trimethyl-silyl derivatives. GC-MS was performed in SCAN mode for stimulants and in SIM mode for synthetic cannabinoids (SC).

For LC-MS/MS and LC-HRMS analyses, urine samples were diluted 1:5 and directly analyzed, while blood samples were purified by DLLME. Hair were incubated in an acidic solution overnight for stimulant NPS, followed by an overnight incubation with methanol for cannabinoids. LC-MS/MS was performed in ESI in MRM mode. Analytes were grouped in three analytical methods with optimized elution gradients and MRM transitions: stimulants, synthetic opioids, SC and metabolites.

LC-HRMS analyses were performed using a benchtop Orbitrap at a resolving power of 100000 FWHM to identify the accurate mass of the analytes. For qualitative screening, each sample was processed by a home-made database, which included the name of the analyte, its molecular formula and protonated accurate mass. This allows at now the screening of over 200 NPS, covering the classes of cathinones, SC, phenethylamines, piperazines, tryptamines, fentanyls, benzodiazepines and many natural NPS. In case of a suspect positive sample, it was subsequently analyzed in CID to study its characteristic fragments. If available the analytical standard, the mass spectra and retention times were compared.

Results

All the methods were validated considering the following parameters: LOD, LOQ, specificity, matrix effect (ME%) and extraction efficiency (LC-MS) or extraction recovery (GC-MS), linearity. For GC/MS methods, LODs vary from 2 to 50 ng/mL for stimulants and from 1 to 5 ng/mL for synthetic cannabinoids in urine and blood. For LC-MS methods, LODs obtained from 1 to 5 ng/mL for urine, from 2 to 30 pg/mg for hair and for blood from 0.05 to 0.2 for fentanyl and its analogues, and from 0.2 to 2 ng/mL for the other substances. DLLME allowed an acceptable ion suppression/enhancement also for complex matrices as blood samples, and ME% ranged from 61 to 110%.

The methods were applied to authentic urine, blood and hair samples, allowing the identification of various NPS. Some examples will be reported for each analytical technique described.

Discussion

The challenge of the screening of NPS in biological fluids requires the development of sensitive, specific and high-throughput analytical methods that, in principle, can be used by laboratories of all levels. We propose different analytical methodologies based on different equipment, which can respond to this need at a different level of cost but with high levels of sensitivity and specificity. The GC-MS technique requires sample extraction and derivatization; the use of DLLME renders this step very rapid and inexpensive. The methods developed allow the screening of stimulants and SC by GC-MS with adequate sensitivities.

Both LC based methods, allow the analysis of urine samples after a simple dilution. Hair digests, in the same way, can be directly injected in the LC-MS systems.

Blood samples require a prior extraction before instrumental analysis. Nevertheless, DLLME preparation allows good sensitivities and acceptable matrix effect.

After pre-treatment, each sample can be screened for a huge number of NPSs, especially by using HRMS technique, in SCAN acquisition mode, which allows the detection of a potential infinite number of analytes, knowing their raw formula, by the use of a home-made database. The confirmation of identity of a suspect analyte can be performed by a further experiment in fragmentation mode.

Conclusions

Different analytical methods were developed for the screening of a huge number of NPSs in biological samples, by employing different typologies of analytical techniques. From a more basic GC-MS method, affordable by all laboratories, to LC-MS/MS, allowing the screening of a wide number of target compounds with high sensitivity, to LC-HRMS, enabling the screening of a potential infinite number of new substances, with a high specificity.
Abstract ID 453
Laminar Flow Mass Spectrometry for the Detection and Quantification of Fentanyl, Norfentanyl, and Acetaminophen in Whole Blood.
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Aim
Fentanyl, a synthetic opioid, has been found increasingly in opioid overdose deaths all over the world. With a lethal dose of only 2 mg in most humans, heroin and other drugs laced with fentanyl pose a growing problem on a global scale. This project focused on developing and validating a method for the detection and quantitation of fentanyl, its primary metabolite norfentanyl, and acetaminophen in human whole blood with the use of laminar flow mass spectrometry. It also evaluated the use of Phospholipid Depletion Cartridges (PLD, Biotage, Charlotte, NC, USA) in extracting compounds from the human whole blood (Equitech Enterprises, Kerrville, TX, USA). These compounds were analyzed using UHPLC (PerkinElmer, Waltham, MA, USA) using a Restek Raptor biphenyl 2.7 micron 100 x 3.0 mm (Restek, Bellefonte, PA, USA) with a QSight® 220 CR LC-MS/MS (PerkinElmer, Waltham, MA, USA) in positive ion mode.

Results
Calibration model, linear dynamic range (LDR), limit of detection (LOD), and limit of quantitation (LOQ) were assessed during method development and validation. All compounds were quantified using a linear model. LOD and LOQ were determined to be 0.5 ng/mL, with LDR of 0.5-500 ng/mL with r2 values of 0.99 or greater.

Discussion

Conclusions
The method was validated following parameters set by ASB Standard 036-Standard Practices for Method Validation in Forensic Toxicology. PLD allowed for an efficient yet effective method for sample extraction for analysis by UHPLC-laminar flow MS/MS.
Abstract ID 468
Evaluation of Sample Preparation Approaches for the Extraction of Amphetamine, Methamphetamine, MDMA and metabolites from Urine prior to GC/MS Analysis.

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Aim
Amphetamine, methamphetamine and Ecstasy continue to be widely abused in many parts of the world with urine analysis continuing to be the most popular approach to determining drug intake. The aim of this poster is to investigate various sample preparation approaches for the extraction of amphetamines from urine. Optimized methods using solid phase extraction (SPE), supported liquid extraction (SLE) and a novel approach of dual mode extraction (DME) will be presented and compared for recovery, reproducibility, sensitivity, calibration, procedural simplicity, solvent consumption, associated assay time and overall cost.

Methods
Negative urine was provided by healthy human donors and spiked with an analyte mix of amphetamine, methamphetamine, MDA, MDMA and MDEA. Amphetamine–D5 was utilised as the internal standard for calibration testing and spiked into the sample prior to pre-treatment. Analyte extraction was investigated using various sample preparation approaches: silica and polymer-based SPE, supported liquid extraction (SLE) and dual mode extraction (DME). Optimized procedures were evaluated for overall performance. Extracts were evaporated at ambient temperature and concentrated if applicable, followed by in-vial derivatization with ethyl acetate (50 µL) and pentafluoropropionic anhydride (PFPA) (50 µL). The samples were capped and heated at 50 °C for 15 minutes then cooled. The samples were once again evaporated at ambient temperature and reconstituted in ethyl acetate for analysis. GC/MS analysis was performed using an Agilent 7890 GC and a 5975 MSD, following sample injection of 2 µL in splitless mode. Chromatography was performed on a Restek Rxi®-5ms capillary column; 30 m x 0.25 mm ID x 0.25 µm using 1.2 mL/min helium. Positive ions were acquired using electron ionization operated in SIM mode.

Results
Optimized silica-based SPE returned clean extracts and typical recoveries in excess of 95%.

The polymer-based SPE approach allowed recoveries of 87-102% with RSD below 5% using full and simplified load-wash-elute extraction protocols. The supported liquid extraction approach allowed recoveries of 97-108% with RSD values below 10%. Dual mode extraction returned recoveries below the other techniques, generally up to 80% with corresponding RSDs below 10%. Calibration curves were constructed from 10-1000 ng/mL with Amphetamine-D5 used as internal standard spiked at 100 ng/mL. Limits of quantitation for both the silica and polymer based SPE approach were determined to be 10 ng/mL or better for all analytes. The supported liquid extraction and dual mode extraction approaches offered LoQ values of 20-45 ng/mL, still well below the SAMHSA/EWDTS recommended cutoff values.

Discussion
Silica-based SPE has historically been used for urinary amphetamine analysis and was the benchmark for this study. Although excellent sensitivity and overall performance silica based SPE suffers from some drawbacks not associated with modern techniques: silica sorbents require extensive phase conditioning, often methanol followed by at least one aqueous buffer for pH control of the sorbent. These steps add time, solvent usage and complexity to each assay. Modern polymer-based SPE such as EVOLUTE EXPRESS contain water wettable components allowing the elimination of phase pre-conditioning. The resulting load-wash-elute protocol provides cost saving in terms of solvent use and disposal. The more retenive nature and higher capacity of polymer-based SPE also allow column size reduction which consequently led to reduced wash and elution volumes. The latter further allows elimination of added concentration steps, depending on technique being employed and elution volumes. Likewise the use of supported liquid extraction allows a streamlined processing load-wait-elute protocol to be used. Simple processing, no waste but the use of water immiscible organic solvents mean evaporation is almost always required. Finally techniques such as dual mode extraction employ a simple acetonitrile precipitation followed by flow through processing. The media scavenged urinary components resulting in an acetonitrile rich elution. Once again simplified processing compared to traditional approaches.

Conclusions
This poster demonstrates a range of approaches for the extraction and cleanup of amphetamine, methamphetamine, MDMA and metabolites from urine. Various sample preparation approaches exhibit the required method performance in terms of recovery, extract cleanliness and sensitivity. However, individual laboratory perspectives in terms of cost, assay time, solvent and reagent use may dictate final choice of technique.
Abstract ID 470
Comparison of Mid-InfraRed, Near-InfraRed and Raman spectroscopy combined with chemometrics to classify and quantify seized cocaine powders.

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Aim
The aim of this study was to compare the performance of three spectroscopic techniques (near-infrared (NIR), mid-infrared (MIR) and Raman) for the rapid identification and quantification of cocaine in powders. An approach based on spectroscopy combined with chemometrics is presented to discriminate cocaine powders from other (drug) powders and to obtain an estimate of the purity.

Methods
A total of 364 seized drug powders were collected during the period 2013 and 2018. All powders were screened using MIR, NIR and Raman spectroscopy. The spectra were pre-processed using standard normal variate (SNV) transformation.

Two chemometric models using Support Vector Machines (SVM) were constructed with each spectral dataset (MIR, NIR and Raman). The spectra were pre-processed using standard normal variate (SNV) transformation.

Two chemometric models using Support Vector Machines (SVM) were constructed with each spectral dataset (MIR, NIR and Raman). A classification model (SVM-discriminant analysis; SVM-DA) was created in order to classify cocaine powders from other powders. A quantification model (SVM regression; SVMR) was built to quantify cocaine in the powders classified as cocaine-positive.

The performances of these 6 calibration models were compared with the reference methods, GC-MS and GC-FID. To evaluate the classification models, the number of false negatives (FN), false positives (FP) and the total classification rate were calculated. For the quantification models, the root mean squared error of prediction (RMSEP), correlation coefficients (R2) and biases were used to evaluate the agreement between cocaine concentrations obtained by SVMR and GC-FID.

Results
The powders without cocaine (n=88) consisted of heroin, amphetamine, methamphetamine, MDMA, ketamine, medicines (sildenafil, methadone and bromazepam) and new psychoactive substances (methylylene and 25I–NBOMe). Also adulterants (ibuprofen, benzocaine, paracetamol, caffeine and phencetin), sugars (sorbitol and lactose) and other diluting agents (starch, sodium hydroxide, milk powder, washing powder and calcium carbonate) were found. The percentage of cocaine in the cocaine street samples (n=276) ranged from 4% to 99%. Levamisole, phenacetin, diltiazem, caffeine, hydroxyzine, boric acid, benzoic acid and lidocaine were found as adulterants.

Due to fluorescence background in Raman spectra, 10 classification samples (coloured powders) could not be measured. These samples were excluded from the classification dataset. However, this limitation was taken into account for the sensitivity and specificity calculations. For the differentiation between cocaine positive and negative powders (SVM-DA models), the best classification (99.8%, 1 FN sample) was obtained with MIR spectra. With Raman and NIR spectra, the classification rate was 99.6% (2 FN samples) and 97.7% (4 FP samples), respectively.

For the quantification of cocaine positive powders (SVMR models), the best results were obtained with NIR spectra. The cocaine content was determined with a RMSEP of 3.79% and a R2 value of 0.97. MIR and Raman showed similar results, with RMSEPs of 6.76% and 6.79%, respectively and both a R2 of 0.90. Biases were comparable for the three techniques: -0.26 (NIR), 0.30 (MIR) and 0.66 (Raman).

Discussion
For the classification of powders (cocaine detected/not detected), the best model (in terms of the lowest misclassifications) was obtained using MIR. The MIR spectra contained richer spectral information. For the estimation of the cocaine content, NIR showed the best prediction performance. The NIR technique had the advantage that a larger area of the sample can be measured.

Conclusions
It could be concluded that for both classification and quantification purposes MIR spectroscopy was pointed out as the most suitable technique for routine application, but also NIR and Raman could be applied.
Abstract ID 481
Detection of drugs of abuse in DBS and DUS collected on a dried matrix spot cardboard cartridge using on-line SPE-HPLC-MS/MS.

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Aim
Biological analysis of drugs of abuse (DOA) is usually performed using either blood by venepuncture or urine, but the use of dried blood/urine spots (DBS/DUS) has grown in popularity in toxicology as an alternative sampling procedure because of the minimal invasive and easy sampling of blood and higher analyte stability for some compounds. A newly designed lateral-flow immunoassay cardboard cartridge (Greencheck, Protzek Biomedizinische Technik GmbH, Lörrach, Germany) containing a filter paper for collection of dried matrix spots (DMS) was provided, and the detection of DOA by LC-MS/MS had to be evaluated.

Therefore, the aim of this project was the development and validation of a one-step extraction and analysis protocol for the 20 most common DOA (cannabinoids, cocaine, opioids and phenylethylamines - parent compounds and metabolites) on these DBS/DUS cartridges.

Methods
The DBS/DUS samples were prepared from liquid venous blood or urine. 20 µL (for blood) or 10 µL (for urine) were pipetted on the filter paper in the cardboard cartridge and left to dry for 2 h at ambient temperature. The whole DBS/DUS was punched out (10 mm i.d.) and transferred into an Eppendorf tube. To each sample, 1 mL of methanol and 10 µL of internal standard were added. After shaking for 15 min and centrifugation for 5 min, the solvent was transferred into a micro-vial. Then, 10 µL of hydrochloric acid (37%) were added and the solvent was evaporated to dryness at 40 °C under a gentle stream of nitrogen before being reconstituted in 100 µL of acetonitrile/water (5:95; v/v). Optimising the detection of cannabinoids, the reconstitution solvent was adjusted to an acetonitrile/water ratio of 20:80; v/v. Thereof, 5 to 20 µL were injected into the on-line SPE-LC-MS/MS system (Sciex 5500 QTRAP® with TurbolonSpray Source ESI+ in Multiple Reaction Monitoring), which consisted of a trapping column (Phenomenex Synergi Polar-RP, 20 x 20 mm) and an analytical column (Phenomenex Kinetex F5 2.5 µm, 30 x 2.1 mm) with a gradient of 2.5 – 95% water/acetonitrile (0.1% formic acid).

Results
For the tested polar DOA, generally good linearity was achieved between 2.5 and 250 ng/mL. Only the opiates 6-MAM, morphine and codeine showed somewhat higher limits of quantitation between 10 and 15 ng/mL. For the more non-polar DOA Δ9-tetrahydrocannabinol (THC), cannabidiol (CBD) and 11-hydroxy-THC (11-OH-THC), good linearity was obtained between 1 and 20 ng/mL, and between 5 and 100 ng/mL for 11-nor-9-carboxy-THC (THC-COOH), respectively. Setting the lowest limit of quantification at 0.5 and 2.5 ng/mL, respectively, challenges regarding meeting the criteria for accuracy and precision still need to be overcome. In general, these intra- and inter-assay criteria were met in the calibration areas and found in a range of 80 to over 100%, with an exception for the before mentioned opiates. Furthermore, extraction recoveries ranged mainly from 90 to over 100%, with lower values for 6-MAM, Morphine, EDDP, THC, 11-OH-THC and THC-COOH. In addition, no significant interferences were observed in blood and urine.

Discussion
The lower recovery values for the cannabinoids may be explained by the composition of the extraction and reconstitution solvent. Although the proportion of acetonitrile already has been increased for the optimized method, it can be supposed that the recovery would be better with even higher amounts. This, however, would lead to a decrease in recovery of the polar analytes. Overall, this method shows a good compromise between all the analytes with reproducible accuracy and precision, but further optimization seems to be necessary.

Conclusions
The new extraction and analysis protocol for the 20 most common DOA (cannabinoids, cocaine, opioids and phenylethylamines - parent compounds and metabolites) on DBS/DUS cartridges has been validated. It was possible to implement only one single protocol for the simultaneous extraction and analysis for polar and non-polar analytes. These results are promising for the introduction of DBS/DUS as alternative sampling method as the recovery is even high enough for the detection of drugs at low concentrations and below “legal limits”. 
A Benchtop NMR approach for the determination of ethanol and methanol in alcoholic beverages in cases of suspected poisonings and drink spiking

Geraldine Dowling

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Abstract

The research has demonstrated the potential for use of the instrument in forensic toxicology teaching in an accredited forensic science programme and in addition to customs/toxicology/forensic laboratory personnel whom are new to NMR interpretation.

Discussion

This approach could be used by forensic laboratories to shorten analyses times for methanol and ethanol in alcoholic beverages. In the case of toxicology laboratories, this technique could be extended to the analysis of other alcohols or the determination of ethanol in beverages to ascertain as to whether or not a drink has been spiked with additional alcohol or other agents and in suspected methanol poisonings cases. Lastly, many laboratories do not have access to highly trained personnel in the field of NMR interpretation however this method was further implemented in a forensic science program as a proof of concept allowing undergraduates to run experiments and to identify how easy it is to use, interpret the data and generate rapid results with very basic NMR training. This proof of concept approach is less expensive than highfield NMR and appealing to customs/toxicology/forensic laboratory personnel whom are new to NMR interpretation.

Conclusions

Overall, analysis of alcohol poisoning cases allows Benchtop NMR to be routinely implemented in forensic toxicology cases regarding drink spiking, methanol poisonings or drug-facilitated sexual assaults. In addition the Benchtop NMR methodology can be used to train forensic scientists, toxicologists, laboratory analysts and forensic science students regarding case interpretation within their own laboratory setting in conjunction with chromatography mass spectrometry methods.

Teaching new innovations across different target audiences is very important if we are to attract and motivate a new generation of forensic toxicologists/scientists/forensic laboratory and customs personnel to study forensic science and solve forensic problems of today as well as into the future.
Abstract ID 485

Phase I metabolic profiling of the synthetic cannabinoid EG-018 by pooled human liver microsomes, CYP isoenzymes, Cunninghamella elegans and urine screening.

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Aim
To examine the phase I metabolism pathways of the carbazole-derived synthetic cannabinoid EG-018 (naphthalen-1-yl(9-pentyl-9H-carbazol-3-yl)methanone), which was first listed in Latvia in 2014, different in vitro approaches were utilized. In addition, screening of two urine samples from proven EG-018 users was performed. Regarding the in vitro approaches, two different microsomal incubation assays (pooled human liver microsomes (pHLM) and selected cytochrome P450 isoenzymes (CYPs)) and one fungal model (Cunninghamella elegans LENDNER, hereinafter abbreviated as C. elegans) were applied. C. elegans is already well-known for its ability to mimic human biotransformation of xenobiotics, including some synthetic cannabinoids. Finally, results were compared with each other, and with literature, in order to examine the applicability of different in vitro assays for in vitro – in vivo extrapolations.

Methods
The microsomal incubation assays consisted of phosphate buffer, MgCl2, SOD, an NADPH generating system (NADP, gluc-6-P and gluc-6-P-dyhydrogenase), EG-018 and preparations of pHLM or selected CYPs (1A2, 2B6, 2C9, 2C19, 2D6, 3A4, 3A5). The EG-018 final concentrations were 102 µM in the pHLM and 10.2 µM in the CYP assay, respectively. Incubation was performed at 37 °C and stopped after 3 hours with ice-cold acetonitrile. Fungal incubation was performed at 37 °C and stopped after 3 hours. Following incubation, the medium was extracted with dichloromethane. After evaporation of the extract, the residue was dissolved in ACN/water=50/50 (v/v) and further extracted via SPE utilizing C18 disk cartridges. Supernatants of the microsomal incubations and urine samples were extracted analogously. In vitro and in vivo metabolic modifications of EG-018 were examined by means of precursor ion scans, as well as measurements in the MRM mode, utilizing an LC-MS/MS system from Agilent Technologies (1200SL HPLC coupled to a 6460A triple-quadrupole) equipped with a Phenomenex Strata Core-Shell Biphenyl column. The LC-eluent consisted of 2 mM ammonium formate buffer (solvent A) and ACN (solvent B), each acidified with 0.1% formic acid. Evaluation of some fragment ions was supported by LC-HRMS (Q Exactive Plus) analysis in cases where LC-MS/MS alone was insufficient for identification.

Results
Microsomal incubation with pHLM yielded 15 metabolites of EG-018 belonging to six different metabolic pathways: monohydroxylation (M1.1-M1.5), dihydroxylation (M2.1-M2.5), carbonylation (M3.1-M3.2), carbonylation and monohydroxylation (M4.1), dihydrodiol formation (M5.1) as well as desalkylation (M6.1). Among them, M1.1, M1.2, M1.5 and M3.1 were the most abundant metabolites found after pHLM incubation. CYPs and C. elegans delivered most of the identified EG-018 metabolites produced by pHLM, along with some further metabolites, e.g. C. elegans was proven to produce the EG-018 N-pentanoic acid. However, further metabolites identified with CYP and C. elegans were not detected in vivo. With reference to their summed metabolite peak abundancies, CYP2C19, CYP2D6, CYP3A5, CYP3A4 and CYP2D6 were observed to contribute most to the microsomal metabolism of EG 018. Urine screening yielded the detection of four metabolites (M1.1, M1.2, M2.4 and M6.1), of which two were recommended as urinary targets (M1.2 and M2.4). Thereby, M1.1 was neglected as a urine target as it constitutes as a shared metabolite with EG-2201 (SF-EG-018), M6.1 was waived due to its low abundance. While M1.1 and M1.2 were shown to be produced by all of the CYP isomers, and M6.1 by four out of seven; M2.4 was only produced by CYP2C19 and CYP3A5.

Discussion
The final comparison of the results revealed that relevant urinary targets were produced by all of the tested in vitro approaches, wherefore all of the tested in vitro approaches were demonstrated to be useful tools for in vitro – in vivo extrapolations regarding the phase I metabolism of EG-018. This was also confirmed by comparison with the previously published data. However, with regard to the C. elegans approach, further investigations will be required to investigate the ability of C. elegans to produce high(er) amounts of EG-018 metabolites, e.g. for further research.

Conclusions
Combined application of various different in vitro models is recommended for exhaustive in vitro metabolism studies, as the models are complementary, and knowledge of the complete spectrum of urinary targets, implemented in screening methods, is substantial. This is particularly important if in vivo extrapolations have to be done for investigation and elucidation of comprehensive metabolic patterns. When testing for EG-018 use, and in need for a differentiation between the use of EG-018 and EG-2201, the M1.2 and M2.4 metabolites are recommended.
Abstract ID 489

Analysis of diazepam and its metabolites in dried blood spots using HPLC–MS/MS.

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Aim
1. Background: Diazepam is the most typical medicine of benzodiazepines, and has been widely used in relieving psychiatric disorder symptoms like anxiety, insomnia, muscle spasm and so on. However, its frequent abuse leads to an increasing number of forensic cases such as suicide, drug-driving or robbery. Traditional detection methods, such as solid phase extraction and liquid-liquid extraction from whole blood or plasma, need long extraction time, high costs and limited location as well, it exists a risk of cheating, which may end up with a completely wrong result. Moreover, liquid-liquid extraction could not extract diazepam, its redox products and glucuronic acid conjugates simultaneously in whole blood based on our preliminary study. While, Dried blood spots (DBS), as a newly-development blood extraction method, needs a very small blood sample volume, but also avoid many disadvantages successfully.

2. Objective: The study was supported by the National Science and Technology special project work (2015FY11400) and National Key R&D Project (2017YEC0803504, 2018YFC0807403), and aimed to develop and validate a method for simultaneous quantification of diazepam and its metabolites in dried blood spots, especially for the conjugated metabolites (nordazepam, oxazepam, temazepam glucuronide and temazepam glucuronide) in dried blood spots.

Methods
1. Methods: 100 µL of whole blood was spotted on a piece of filter paper and dried for at least 2 h at room temperature before the paper around the blood spots was cut away and the analytes in the blood spot were extracted with 500 µL methanol/water mixture (8:2, v/v). After vortexing, 10 µL of 1 µg mL−1 diazepam- d5(IS) was added to the extract. The extracting method including sonication for 15 min and centrifuging for 30 min at 13000 r/min, - 4 °C. The solution was then dried under nitrogen gas at 37°C, and reconstituted with 100 µL LC - MS/MS initial mobile phase ( 10 % acetonitrile in water). Finally, the 10 % acetonitrile in water were filtered by a thick membrane (2.2 µm) and 5 µL was injected into the LC–MS/MS system ( Agilent LC-1260 HPLC system and 6460 tandem mass spectrometer ). The mobile phase was mixed by solvent A (0.1 % formic acid in H2O) and solvent B (0.1 % formic acid in acetonitrile). The column was held at 40 °C and eluted for 7.0 min in total at a flow rate of 0.4 mL/min with a gradient of 90% A (0-3.8 min), 10% A (3.8-4.5 min), 90% A (4.5-7.0 min). Electrospray ionization (ESI) was performed in positive mode, data was recorded in multiple reaction monitoring (MRM) mode.

Results
1. Results: The method established made all of the analytes separated well and there was no endogenous peak coeluted with analytes. Lower limits of detection (LLOD) of diazepam and nordazepam, oxazepam, temazepam, oxazepam glucuronide and temazepam glucuronide in dried blood spots were05, 0.5, 0.05, 0.5, 1 and 0.05ng/mL; Lower limits of quantification (LLOQ) were 0.1, 1, 0.1, 1, 2 and 0.1ng/mL. The liner range were 1-100ng/mL for diazepam and oxazepam, 5-100ng/mL for nordiazepam, temazepam and temazepam glucuronide, 10-200ng/mL for oxazepam glucuronide, and the correlation index were greater than 0.99 for all analytes. The results of matrix effect were 0.87, 0.77, 1.13, 0.95, 1.20, 0.94, recovery were 62.06%, 46.31%, 64.57%, 53.55%, 55.74%, 50.72%. Accuracy and precision (intra- and inter-assay) were satisfied with method validation criteria, precise within 15% and 20%(LLOQ).

Discussion
Discussion: DBS is a newly-development blood extraction method used in forensic field with the development of highly precise instruments like LC-MS/MS. It has many advantage over plasma or whole blood due to its small blood sample volume, it can analyze drugs with less than 30 µL of blood compared with at least 500µL of existing extraction methods. In view of a lower concentration of conjugated metabolites in whole blood, 100 µL blood was used to analyze diazepam and its metabolites in blood. Several methods for diazepam and its metabolites quantification in hair, plasma, whole blood or urine were published. The detection of diazepam or some of its metabolites (nordiazepam, temazepam, oxazepam) in dried blood spots were reported as well. However, a method for simultaneous quantification of diazepam and its metabolites in dried blood spots, especially for the conjugated metabolites(temazepam glucuronide and oxazepam glucuronide), has not been found. The study established a method extracting and detecting diazepam and its redox production as well as conjugated metabolites in DBS, simultaneously, which is more sensitive, and meet with the criteria set by U.S. Department of Health and Human Services, Food and Drug Administration (FDA), 2018.

Conclusions
1. Conclusion: We developed a method that is somewhat fast and reliable. The assay might be used to detect the concentration of diazepam and its metabolites in dried blood spots for drug screening.
**Abstract ID 496**

**Drug screening in the DUID context by using the Toxtyper™LC-MSn: preliminary evaluation of its application to serum samples.**

**Rossella Gottardo**

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**Aim**

The toxicological approach in the Driving Under Influence of Drugs (DUID) context needs a high sensitivity screening including as many compounds as possible, in order to limit the number of false negatives. Indeed only “positives” at the screening are followed by confirmation. In this frame, immunological methods are generally used, while GC-MS or LC-MS are applied as confirmation techniques. However, the expanding number of new drugs far exceeds the identification possibilities of the current immunoassays, posing a still unraveled problem in the delicate phase of preliminary drug identification. On the other hand, the alternative approaches based on High Resolution mass Spectrometry at present require complex and expensive instrumentation and highly trained personnel. Toxtyper™ (Bruker Daltonics, Bremen, Germany) is an LC-MS instrument in which ultra-high-performance-liquid-chromatography (UHPLC) is coupled with a high speed Ion Trap mass analyser (IT-MS). The instrument is provided with ready-to-use assay protocols, which include UHPLC and MS set-ups, as well as algorithms for automatic report generation. Although the analytical performances of Toxtyper™ have already been tested in the determination of medicinal drugs in blood [Kempf J et al., Forensic Sci Int. 2014;243:84-9] and of drugs of abuse in oral fluid and urine [Ott M et al. Clinical Mass Spectrometry 2017 4–5:11–18; Plecko T et al. Ther Drug Monit. 2018; 40:642-648], to the best of our knowledge, they have never been verified for the general drug screening in serum in the DUID context.

**Methods**

After protein precipitation of 200 uL serum, samples were analysed on the Toxtyper™ LC/IT-MS system following the instructions provided by the manufacturer. To investigate the analytical performances of the instrument, three pooled serum samples were fortified with a selected drug panel (n= 55) including 17 opioids/opiates, 3 cannabinoids, 17 benzodiazepines/Z-drugs, 3 barbiturates, 11 stimulants (6 amphetamines and analogues, cocaine and metabolites, methylene) and 4 psychedelics at three different concentrations in the ranges of DUID interest for each compound.

The analytical performance was evaluated by analyzing the three pooled serum samples at the three different concentrations using different ionization techniques, i.e. electrospray (ESI), atmospheric pressure chemical (APCI), and heated-ESI (Ion Booster™). The identification capability was verified by using three different library search approaches: the Drug of Abuse (DOA) and the Toxtyper libraries provided by the manufacturer and the commercially available Maurer/Wissenbach/Weber (MWW) library, respectively. The criteria of identification included purity above 850/1000 and deviation of ± 0.3 min from the target retention time.

The influence of temperature vaporizer of the Ion Booster on the signal intensity for the three pooled serum samples at the three different concentrations was evaluated in the temperature range of 200-450°C.

The agreement between the results from Toxtyper and the confirmatory techniques currently in use at the University of Verona (GC and LC-MS) was investigated by analyzing 100 real serum samples randomly chosen from those routinely analysed in the frame of DUID investigation.

**Results**

The data of analytical sensitivity, evaluated as the percentage of identified substances, each of them at its lowest concentration, by using the different ionization techniques and the different libraries can be summarized as follows.

**ESI SOURCE**

DOA search library: 72.7%  
Toxtyper search library: 56.4%  
MWW: 50.9%

**APCI SOURCE**

DOA search library: 67.3%  
Toxtyper search library: 43.6%  
MWW: 36.4%

**ION BOOSTER**

DOA search library: 85.5%  
Toxtyper search library: 70.9%  
MWW: 78.2%

In order to further study the analytical performances of Ion Booster, an evaluation of the influence of temperature vaporizer on the signal intensity was also performed showing that this parameter looks to affect the signals of the different substances in a variable way. The results will be shown in detail in the presentation.

**Discussion**

The best results, in terms of analytical sensitivity, were obtained by using Ion Booster as the ionization source and DOA as search library approach.

These data are consistent with those reported in the literature, where Ion Booster™ proved to provide an increased signal in comparison to conventional ESI [Huppertz LM et al., Journal of Mass Spectrometry 2014, 49:117-127]. DOA library consistently gave better analytical sensitivity as compared to the other 2 libraries, probably because the DOA library method is based on a targeted screening approach on a smaller number of molecules.

It is important to mention that neither ESI nor Ion Booster were able to produce, in the case of cannabinoids, detectable signals in the tested concentration range (1.25-25 ng/mL). On the other hand, APCI, even if overall less sensitive than the other two sources, was able to produce, in the case of cannabinoids, a detectable signal at 6 ng/mL.

**Conclusions**

The present study highlights the suitability of Toxtyper IT-MS for drug screening in serum with a sensitivity compatible with the needs of the DUID context for all the tested compounds but, so far, not for cannabinoids.
Abstract ID  503
Abused drugs, heavy metals and microbiological analysis in Izmir Küçük river.
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Aim
International attention is focusing more and more on the growing issue of drug abuse analysis. Water based studies provide in a social aspect realistic, reproducible, objective determination of illegal substances and their levels. Izmir Küçük Menderes River is intensive agricultural and industrial activity is above Turkish general level. The aim of this study is to analysis abused drugs, heavy metals and microbiological analysis in surface waters samples taken from Izmir Küçük Menderes River.

Methods
Microbiological, heavy metals and illegal substances (morphine, codeine, cocaine, benzylecgononine, 3,4-methylenedioxy-N-methylamphetamine (MDMA), amphetamine and 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH)) analysis were performed in surface waters (1000 ml) collected from 4 different stations from Izmir Küçük Menderes River in April 2019. The water samples were taken in amber glass bottles and brought to the laboratory within 24 hours by cold chain and analysed at room temperature. Heavy metal analyses were performed by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) according to Turkish Standards Institute TS EN ISO 17294-1/2 method: ‘Water quality-Application of ICP-MS: Microbiological analysis of Coliform bacteria and Escherichia coli (E.coli) analysis were performed according to TS EN ISO 9308-1 method and Enterococcal bacteria were performed according to TS EN ISO 7899-2. For drug analysis, before liquid chromatography tandem mass spectrometry (LC-MS/MS) analyzing the parameters (flow rate, mobile phase ratio and injection volume) were optimized. All water samples were filtered to remove solid particles. For sample preparation; solid phase extraction (SPE, Oasis MCX SPE) method was used, cartridge was conditioned with 5 ml of methanol, 5 ml of deionized water and 5 ml of ultrapure water (pH:2), respectively. 5 ml of sample was passed through the cartridge. After drying, 6 ml of methanol and 6 ml 2% ammonia solution in methanol were passed through for elution. Then, eluents were evaporated under nitrogen gas. Residues were dissolved in 1 ml water: methanol (75:25,v/v) solution and filtered. Samples were analysed by LC-MS/MS TSQ Quantiva Triple-Stage Quadrupole Mass Spectrometer with Selected Reaction Monitoring (SRM) mode. The MS source parameters: mass scan range is 10-1800, ionization mode is heated electrospray ionization (HESI), spray voltage is 3500V. The developed method was validated according to the international guideline (SWGTOX).

Results
The results of the microbiological analysis were detected above the limits according to the spring water notification. For heavy metal (Na-Mg-Al-P-Cr-Mn-Fe-Ni-Cu-Se-Cd-Sn-Hg-Pb) analysis, the results for arsenic and nickel were above 1 µg/L. The validated method was selective, calibration range was between 10-500 µg/L and R2 values were greater than 0.99 for the abused drugs. The mean relative standard deviations of intra-day repeatability were calculated for amphetamine 3,95-4,10, for cocaine 11,99-5,29, for benzoylcegonine 2,79-3,67, for codeine 8,16-5,81, for MDMA 8,9-2,99, for morphine 14,41-7,39 for THCCOOH 2,19-6,19 for the concentration of 25 µg/L and 100 µg/L, respectively. The limit of detection (LOD) varied between 3,46 and 6,79, the limit of quantitation (LOQ) value was 25 µg/L for analysts. The recovery values of the method were ranges between 74,17% and 95,91%. There was no positive drug result above our LOQ value. However, for cocaine, benzylecgonine and amphetamine were detected semiquantitative/qualitative (above LOD) at four different stations from the River.

Discussion
According to microbiological analysis results the presence of E. coli or faecal coliform bacteria in any sample was indicative of direct or indirect fecal contamination to river water. This is a sign of the mix of sewage water to river water. Lower LOQs could be obtained by selecting a suitable LC column for getting better sensitivities. When we reach lower LOQ levels, results can be accepted as a quantitatively positive. However, our column selection did not yield reproducible results below LOQ value.

Conclusions
The use of analytical methods for the detection of abused drugs use from surface and wastewater is very important. In this study, drug abused analysis was performed for the first time in the river waters of Izmir Region. At different stations, we detected cocaine, benzylecgonine and amphetamine semiquantitative results for which were above the LOD values. Drug concentrations can be affected from the water life factors such as, contaminants, enzymes and bacteria etc., therefore should be evaluated in holistic approach. In addition, it is observed that there is no regulation for the analysis and reporting of illegal substances in waters in our country. Having a national guideline compatible with international regulations is important for evaluating illegal substance use.
Abstract ID 504
Solid deposition GC-FTIR and LC-FTIR as highly discriminating techniques for forensic toxicology applications.

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Aim
Over the past years, the coupling of gas (GC) and liquid chromatography (LC) to Fourier-transform infrared spectrometry (FT-IR) has been pursued by means of different types of interfaces, to achieve specific detection and/or identification of sample constituents. Solute-deposition techniques offer inherent advantages over flow-cell technology and spray-type interfaces, in that they provide much better sensitivity and enhanced spectral quality.

A novel desolvation technology is presented, enabling for total flexibility in the coupling with front-end GC and LC separation in a fully automated fashion, and allowing to collect high-resolution transmittance IR spectra in real time and with post-run rescans. Applications of online GC-FTIR and LC-FTIR are illustrated, as effective tools in forensic analysis of drugs. These hyphenated techniques are compelling in providing each compound’s unique fingerprint, making it quick and easy to identify each peak by comparison to the extensive solid-phase IR libraries. Moreover, they fill a gap in identification by mass spectrometry (MS), in discriminating geometrical isomers and diastereomers and closely related molecules.

Methods
After front-end separation, the outlet of a GC (through a heated transfer line) or LC column (through a solvent removal interface) deposits eluents as a continuous track of sample, and acquisition of solid-phase transmission spectra occurs through cryogenically-cooled ZnSe sample disc. The built-in interferometer simultaneously captures a set of time-ordered IR spectra from the deposit track (typically, collected at 0.3 s intervals), and Furthermore allows for post-run data collection, through re-analysis of deposited samples. The width of the deposit track and the IR beam focus point are matched at around 100 mm, allowing for good quality spectra to be obtained down to μg or ng amounts of analytes.

Results
High efficient chromatographic separation was obtained by capillary GC columns or LC columns based on partially porous particle technology, affording high peak capacity and excellent reproducibility of peak retention times/areas. Afterwards, the most sensitive IR detection was achieved, by matching the width of the analyte deposits to the microscope detector area (in the 100-200 μm range), and the signal-to-noise ratio further increased by signal averaging. Chemical identification exploiting the unique FTIR specificity proved well suited to reduce uncertainty in the identification of structural and positional isomers, as well as stereoisomers, enabling to readily differentiate closely related compounds. Some applications including the analysis of amphetamines, NBOMes, cathinones, fentanyl and synthetic cannabinoids will be shown.

Discussion
The results indicates that the sample deposition interface enables to overcome major limitations associated to the use of flow-cell technologies in LC and light-pipes in GC, mainly related to the rather poor detection limits which have precluded so far their application to determination of minor constituents of mixtures. Cryogenic temperature control of the sample disc provides greater resolution than that obtainable with traditional light-pipe and flow-cell devices, by minimizing the area of the deposited analyte. Solid-phase spectra obtained from a thicker sample layer afford greater sensitivity, where gas phase detectors deliver little useful information. In addition, spectra obtained using ZnSe closely resemble conventional KBr disc transmission spectra and attenuated total reflection (ATR) IR spectra, thus spectral libraries and search programs may be used for identification purposes, enforcing the reliability of IR as detection technique.

Conclusions
The DiscovIR interface and technology allow for the GC-FTIR and LC-FTIR couplings to be implemented effectively and in a very versatile manner, by posing virtually no limitations in chromatographic run times, GC temperatures, and LC mobile phase components. Temperature-controlled, vacuum deposition method ensures that the results are accurate and reproducible.

The direct linkage of high resolution chromatography and high sensitive solid-phase IR spectroscopy provides a single automated technique to fill the requirements of the guidelines put forth by SWGDRUG (Scientific Working Group for the Analysis of Seized Drugs), by the use of multiple independent identification techniques. Major benefits consist in reduced uncertainty in identification of isomers, increased sensitivity for the analysis of traditional illicit drugs, new psychoactive substances and pharmaceuticals of toxicological interest, suitability to manage heavy workloads of forensic toxicology laboratories.

Instrumentation: DiscovIR-GCTM and DiscovIR-LCTM deposition and detection systems, HyphenTM LC solvent removal interface (Spectra Analysis Instruments, Inc., Marlborough, MA, USA).
Abstract ID 510
The Qualitative and Quantitative Analysis of Synthetic Cannabinoid Receptor Agonists in Infused Papers within Prison Settings - A Temporal Study 2018-2019.
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Aim
In the UK, synthetic cannabinoid receptor agonist (SCRA) use is most prevalent within the rough-sleeping community and in prison populations. In Scotland, since the introduction of the UK-wide Psychoactive Substances Act 2016,SCRAs are almost exclusively used in prisons with little use in the wider community. Prisons therefore act as an early warning system for the emergence of new SCRA compounds and novel modes of use. This research developed qualitative and quantitative methods for the detection of SCRAs on infused paper and herbal material and explores the utility of screening non-judicial samples seized from Scottish prisons for monitoring and intelligence purposes. In the longer-term it provides intelligence to rapidly investigate potential causes of changes in user behaviour and the observed effects of abused substances which may be caused by changes in the substances present and/or changes or unpredictability of concentrations in consumed doses. This pilot study covers the analysis of seized infused papers from 2 Scottish prisons (out of a total of 15 Scottish prisons) and in future will be expanded to cover a greater number of establishments.

Methods
Qualitative screening of seized samples suspected to contain controlled substances was carried out using gas chromatography-mass spectrometry (GC-MS). For SCRA infused papers, 1 cm² paper sub-samples collected from two areas were extracted using 250μL methanol to provide information on both major and minor components for profiling purposes and to provide samples for chiral screening. SCRA identity was confirmed by comparison with reference standards where available or mass spectral databases and by orthogonal analysis using ultra-performance liquid chromatography with quadrupole time of flight mass spectrometry (UPLC-QToF-MS) in low fragmentation high resolution accurate mass mode (TOF-MS) and tandem (MS/MS) modes. A quantitative GC-MS method for the analysis of AMB-FUBINACA, 5F-MDMB-PINACA (5F-ADB), 4F-MDMB-BINACA, SF-MDMB-PICA, and AMB-CHMICA was developed, validated, and applied to SCRA infused papers and herbal materials. Herbal materials (triplicate 10mg aliquots) and infused papers (3mm diameter punch-sampled sections) were sequentially extracted three times by ultrasonication in 75:25 dichloromethane:methanol with an extraction efficiency of >99%. Samples were then diluted into the calibration range using an internal standard (tridecane) prior to analysis.

Results
From 1 June 2018 to 1 May 2019, 244 seized paper samples from 2 Scottish prisons suspected of being infused with controlled substances were analysed and 30% (73 samples) were positive for at least one SCRA with a clear evolution of the SCRAs present over time. Of the positive samples, 56% (41 samples) contained 5F-MDMB-PINACA (5F-ADB) ranging in concentration from 0.3 ± 0.008 to 1.9 ± 0.06 mg/cm²; 29% (21 samples) 5F-MDMB-PICA ranging in concentration from 0.07 ± 0.002 to 1.3 ± 0.04 mg/cm²; 8% (6 samples) 4F-MDMB-BINACA ranging in concentration from 0.03 ± 0.001 to 2.8 ± 0.08 mg/cm²; 4% (3 samples) AMB-FUBINACA, one of which had a concentration of 1.3 ± 0.04 mg/cm²; 1% (1 sample) AMB-CHMICA ranging in concentration from 1.3 ± 0.06 to 2.1 ± 0.1 mg/cm²; and 1% (1 sample) an unidentified SCRA. 14% (10 samples) contained multiple SCRAs with one found to contain four: 5F-ADB (major), AMB-FUBINACA, SF-MDMB-PICA and CUMYL-4CN-BINACA. Two AMB-FUBINACA sample were also positive for 4F-PHP, a synthetic cathinone. Of the 5F-MDMB-PICA samples, 81% (17 samples) also contained a tentatively identified fluorinated PICA impurity as a minor component (0.4-4.0% of 5F-MDMB-PICA peak area), 43% (9 samples) contained a tentatively identified SCI-MDMB-PICA impurity as a minor component (0.3-3.1% of 5F-MDMB-PICA peak area), and 38% (8 samples) contained both impurities. Two 4F-MDMB-BINACA samples also contained a tentatively identified 4CI-MDMB-BINACA impurity as a minor component (0.3-1.26% of 4F-MDMB-BINACA peak area). All AMB-FUBINACA samples contained EMB-FUBINACA as an impurity (0.21-0.27% of AMB-FUBINACA of peak area) and 10% (4 samples) of 5F-MDMB-PINACA samples contained trace amounts of a tentatively identified SCI-MDMB-PINACA.

Discussion
A timeline of the emergence of different SCRA compounds with concentrations was established. SF-MDMB-PINACA (5F-ADB) and AMB-FUBINACA dominated until December 2018; 5F-MDMB-PICA was detected from November 2018; 4F-MDMB-BINACA, one of the latest SCRAs to emerge on the market, was detected from February 2019; and one AMB-CHMICA was detected in March 2019. This demonstrated that, as expected, following the ban of eight SCRAs in China on 29 August 2018, including AMB-FUBINACA and 5F-ADB, alternative or newly emerged SCRA compounds (e.g. SF-MDMB-PICA and 4F-MDMB-BINACA) increased in prevalence. This demonstrates the influence of international legislative controls in source countries, rather than local legislation on the prevalence of individual SCRAs detected in prisons.

Conclusions
A method for the qualitative and quantitative analysis of SCRAs in herbal material and paper was developed, validated and successfully used to screen non-judicial samples seized from Scottish prisons. The screening of samples has clearly identified a shift in the SCRA prevalence from 5F-MDMB-PINACA and AMB-FUBINACA to 5F-MDMB-PICA and 4F-MDMB-BINACA in prisons. Methods will be developed further to study heterogeneity of SCRAs across infused paper samples and to determine the comparative risk/harms of individual samples as they are consumed by users.
Abstract ID 522
Development a chemometric method in urine for new designer psychoactive hallucinating substance: 5-MEO-MiPT.

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Aim
One of the new tryptamine derivative drugs is 5-methoxy-N-methyl-N-isopropyltriptamine (5-MeO-MiPT) which is psychedelic and hallucinogenic drug, chemically related to the naturally occurring tryptamine and also known as Moxxy. When new substances appear on the market, only little is known about their mode of action, metabolism, and toxicity, their acute and long-term effects or even their addictive potential making the assessment of potential harms in humans challenging. For this reason; rapid, reliable and easy 5-MeO-MiPT analysis of in biological samples has become increasingly important by using chromatographic methods. The aim of this study was to investigate major effective parameters in the 5-MeO-MiPT analysis and to develop a chromatographic analysis method in urine samples.

Methods
In this study, synthetic urine samples were prepared and 5-MeO-MiPT analyzed with mix mode solid phase extraction (SPE) cartridge (Oasis HLB Cartridges, Waters) by gas chromatography mass spectrometry (GC-MS, Thermo Finnegan ISQ MS). GC-MS analysis was conducted capillary column (HP-SMS; length, 30 m; film thickness 0.25 mm; diameter, 0.25 mm; Agilent technologies) using helium as the carrier gas. MS analysis was carried out using electrical ionization (EI) mode and 50-500 m/z range were scanned. For sample preparation; cartridge was conditioned with 2 ml of methanol, 2 ml of deionized water, respectively. 2 ml of sample was passed through the cartridge. 2 ml of 5% methanol in water was used for washing cartridge and 3.5 ml acetone:methanol (70:30, v/v) was used for elution. Then, eluents were evaporated under nitrogen gas stream. Residues were dissolved in 100 μL ethyl acetate solution. In chemometric method, firstly Plackett-Burman design that is number of levels is two (minimum and maximum), were used for determine the number of factors influenced the analytical responses and efficient procedures. To use generators for 7 factors (sample volume, hydrolysis agent volume, hydrolysis temperature, hydrolysis time, conditioning volume, washing volume and elution volume) were performed 8 experiments. The most effective three factors were determined as sample volume, hydrolysis temperature and elution volume. These major effective three factors were applied to central composite design (CCD) and results were evaluated with surface response methodology. CCD have 5 levels (-α, -1, 0, +1 and +α points) and 20 experiments. The analytic method was validated for selectivity, linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, between intra-day repeatability, stability and carry over parameters according to the international guideline (SWGTOX).

Results
In this study, the most effective SPE conditions were found to be sample volume, hydrolysis temperature and elution volume for chemometric screening analysis 5-MeO-MiPT in synthetic urine samples. Optimum values for these three parameters were calculated by surface response methodology and the results were determined 1 ml, 30°C, 3,5 ml, respectively. The optimized method was validated for selectivity, linearity (linear between 25-500 ng/ml), limit of detection (LOD 5,63 ng/ml), limit of quantitation (LOQ 18,75 ng/ml) accuracy (43,01%-101,47%), between intra-day repeatability (at 100 ng/ml concentration, 3 parallel, 4,43% RSD), stability and carry over parameters.

Discussion
The increasing diversity of new psychoactive substance, hallucinogenic effects such as 5-MeO-MiPT, thus making it difficult to analyze, because of their availability, illegal substances are becoming widespread. Natural tryptamines have been used by mankind for millennia, but new tryptamine derivatives have been replacing the consumption of traditional hallucinogens, not only for their strong activity, but also due to legal voids that frequently permit their decriminalized trade. To be able to determine these substances in urine become more important for toxicological analyzes. In a short period of time, it is very important to make less cost analysis and also to find the most effective results. For this reason, chemometric screening designs are important for determining factors in analytical experiments also the generation of large amounts of information from a small number of experiments and the possibility of evaluating the interaction effect between the variables on the response. It is also an analytical advantage to determine 5-MeO-MiPT by GC-MS without use any derivatizing agents.

Conclusions
With this accomplished chemometric study performed in the 5-MeO-MiPT, optimum SPE conditions were determined. Further it should be aimed to make and disseminate for 5-MeO-MiPT metabolites. Multiple response optimization using desirability functions have until now had its utilization limited to the chromatographic field. However, its principles can be applied to the development of procedures using various analytical techniques, which demand a search for optimal conditions for a set of responses simultaneously. 5-MeO-MiPT and other tryptamines analyses is very important for forensic toxicologically, and it is good choice to make by time consumer, practical reliable, cost-effective and reproducible chemometric method.
Abstract ID 524
Analysis of the main components of ayahuasca and their cytotoxicity in dopaminergic cells.
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Aim
Ayahuasca is a psychoactive beverage prepared traditionally from a mixture of the leaves and stems of Psychotria viridis e Banisteriopsis caapi, respectively. It was originally consumed by indigenous Amazonian tribes for ritual and medicinal purposes. Over the years, its use has spread to other groups/populations as a source of personal growth and spiritual connection. Also, the recreational use of the isolated compounds has become prominent. The main compounds of this tea-like preparation belong to the alkaloid family and are N,N-dimethyltryptamine (DMT) and β-Carbolines: harmine, tetrahydroharmine and harmaline. The latter are monoamine-oxidase (MAO) inhibitors, thus allowing DMT to exert its psychoactive and hallucinogenic effects on the central nervous system (CNS). Although consumers defend its use, its metabolic effects and those on the CNS are not fully understood yet. The majority of studies regarding the effects of this beverage as a whole or the individual compounds are based on in vivo, clinical trials or even on surveys. Therefore, it is of utmost importance to further investigate the effects of Ayahuasca compounds using in vitro models.

The present work focused on evaluating the in vitro toxicity caused by DMT, as well as the previously mentioned β-Carbolines on N27 rat dopaminergic neurons. Moreover, the toxicity of harmalol, the main metabolite of harmaline, was also evaluated. Alongside, an analytical method was developed to identify such compounds on five varieties of available commercial teas (Psychotria viridis, Banisteriopsis caapi; Peganum harmala; Mimosa Hostilis and DC AB).

Methods
Cytotoxicity was evaluated by means of the CCK-8 assay. The range of concentrations used to test DMT was from 0.00008 to 1 μM, from 0.00512 to 80 μM for harmaline and from 0.00064 to 10 μM for the remaining compounds. The incubation time was tested between 1 h and 24 h. Likewise, all of the teas prepared were tested in cells. Additionally, the quantification of total protein of the cells was also evaluated, testing the highest and lowest concentrations used on the cell viability assay.

For this study, a gas chromatographer model HP7890B coupled to a mass spectrometer model 5977A from Agilent Technologies was used. The column was constituted of 5% phenylmethylsiloxane (30m × 0,25 mm; 0,25 μm i.d.) from Agilent Technologies. Data was acquired in the selected ion monitoring. Solid Phase extraction (Oasis MCX3cc) was used as sample preparation technique.

Results
As a whole, results showed that compounds are cytotoxic in a dose-dependent manner, but the same did not happen concerning time.

Harmalol is capable of considerably diminishing cell viability for concentrations higher than 2 μM. At higher concentrations (10 μM), both harmine and tetrahydroharmine demonstrated to be cytotoxic. Furthermore, harmaline exerted toxicity in N27 cells with a concentration of 80 μM. However, when cells were exposed to DMT alone the results did not show any statistical significance. Conversely, when cells were exposed to the five different teas and to two tea mixtures of P. viridis with P. Harmala or B. caapi, cell viability decreased considerably when compared to control cultures.

Concerning the analysis of the compounds present in commercial teas, four different extraction solvents were tested in order to maximize extraction yields. The first with dichloromethane, isopropanol and ammonium hydroxide [80:20:2]. The other three used methanol, isopropanol and ammonium hydroxide in different proportions: [40:60:0.25], [65.5:32.5:2] and finally methanol and ammonium hydroxide [98:2]. The elution with dichloromethane, isopropanol and ammonium hydroxide [80:20:2] provided the best results for sample extraction.

Discussion
The results obtained from the total protein quantification were consistent with the CCK-8 results, since the assays for the highest concentrations, total protein was affected, meaning that protein production could have been affected by the effect of the compounds. Moreover, CCK-8 results obtained for the five teas and the two mixtures suggest that the synergetic effect of compounds present in each plant exert neurotoxicity. Regarding analytical quantification, P. viridis presented the highest DMT content, while P. harmala presented the highest content of β-Carbolines.

Conclusions
This is the first study to investigate cytotoxicity of Ayahuasca compounds and commercial teas on dopaminergic cells. This is significant since one of the organs affected by the intake of such substances is the brain. Furthermore, not all of the teas include DMT, the same happens for some of the β-Carbolines. Nonetheless, more studies would be important to assess chronic effects of the compounds and the cellular mechanisms behind their cytotoxicity, even the determination of these compounds in biological samples.
Detection and Quantitation of Cannabidiol and Δ(9)-Tetrahydrocannabinol in Oral Fluid of a Therapeutic-Use Cannabidiol Donor Using UHPLC-Laminar Flow-MS/MS.

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Aim
Cannabidiol (CBD) is one of over 80 active cannabinoids found in Cannabis Sativa and is the second most abundant cannabinoid derived from the plant following Δ(9)-Tetrahydrocannabinol (THC). As opposed to THC, CBD does not appear to have any psychotropic effects. Legislation regarding the therapeutic or recreational use of CBD varies, however many individuals use CBD products for management of seizures, anxiety, insomnia, and more. During the extraction of CBD from plant material, THC may be co-extracted. Therefore, screening and quantitating potential THC levels in individuals using CBD products is important in instances where the legality of use of THC does not match that of CBD.

Methods
This project evaluated the detection and quantitation of CBD, THC, and two primary metabolites in oral fluid samples of a therapeutic-use cannabidiol donor using Supported Liquid Extraction (SLE) and subsequent testing by PerkinElmer QSight® 220 CR LC-MS/MS (PerkinElmer, Waltham, MA) in positive ionization mode using a PerkinElmer® Brownlee C18 2.1x50mm (2.7 μm) column. A method was developed for the detection and quantitation of THC, CBD, 11-hydroxy-Δ(9)-THC, 11-nor-9-carboxy-Δ(9)-THC, Δ(9)-THC-d3, and 11-hydroxy-Δ(9)-THC d3 (Cayman Chemical, Ann Arbor, MI, USA). All samples, calibrators, and quality controls were prepared by spiking certified reference standards into synthetic oral fluid (UTAK, Valencia, CA, USA). Calibrators were prepared at 1, 2.5, 5, 10, 20, 30, and 50 ng/mL, to evaluate the calibration model and to identify the limit of quantitation (LOQ) and limit of detection (LOD) with quality controls analyzed at 3, 15, and 40 ng/mL. Samples were prepared in 5% glacial acetic acid (Acros, New Jersey, USA). SLE was performed using ISOLUTE SLE+ 1mL columns (Biotage AB, Uppsala, Sweden) with elution in hexane:ethyl acetate:MBTE (80:10:10), followed by evaporation. All samples were reconstituted in 100μL of 0.1% formic acid (Sigma-Aldrich, St. Louis, MO, USA) in DI water:0.1% formic acid in acetonitrile (70:30). Validation parameters were assessed using ASB Standard 036-Standard Practices for Method Validation in Forensic Toxicology, including carry over, LOD, LOQ, linear dynamic range, internal standards interferences, and calibration model.

Results
All compounds were quantified using linear calibration models. The linear dynamic range was determined to be 1 to 50 ng/mL with a LOQ of 1 ng/mL and a LOD of 0.5 ng/mL. Carryover was assessed by running a double blank following a sample spiked at 50ng/mL with no analytes observed. Oral fluid samples spiked with only deuterated internal standards were used to determine any potential interferences and none were observed. Total run time including equilibration was eleven minutes.

Discussion
The donor samples were collected at several timepoints during the oral administration of an 8mg dose of CBD. These timepoints included prior to administration, 30 minutes post administration, 45 minutes post administration, 60 minutes post administration, 90 minutes post administration, and 120 minutes post administration. CBD was quantified within the samples from below LOD to >50 ng/mL, above the highest calibrator. THC was quantified within the samples from below LOD to 0.7 ng/mL. Metabolites were not detected above the LOD.

Conclusions
Overall, the use of laminar flow mass spectrometry was effective in detecting various cannabinoids in oral fluid samples following SLE sample extraction.
Abstract ID 545
A low voltage paper spray ionization Q-TOF method for screening of NPS in street drug blotter samples.

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Aim
Screening methods using paper spray Q-TOF provide useful data for forensic laboratories because they allow non-targeted analysis, serving as an essential tool in the investigation of known and unknown compounds. This technique can be suitable to identify the new psychoactive substances (NPS) in street drug blotter samples. Generally, gas or liquid chromatography coupled with mass spectrometry are used for the identification of NPS drugs. These methods are time consuming and requires a sample pretreatment that can easily destroy the sample integrity. The paper spray ionization method exclude the need of complex sample pretreatment resulting in a faster analysis suitable for rapid screening purposes. Usually high-voltage (3-5kV) is applied generating an electrospray-like ionization on the paper tip. The aim of this study was to develop a low-voltage paper spray ionization technique and its application in the detection of NPS in street drug blotter samples.

Methods
Seized blotter samples were analyzed by an in-house constructed low-voltage paper spray source coupled to a Q-TOF mass spectrometer (micrOTOF-Q III, Bruker Daltonics, Germany). The samples were cut in triangles with dimensions of 15 x 15 mm (base x height) and placed in a ground electric connection with a power supply comprised of a 9V battery. Each sample was directed toward to the mass spectrometer inlet in a distance of 3 mm. An aliquot of 100 µL of methanol was added to the sample and a low voltage was applied providing a field strength that is high enough to eject the target ions to the glass capillary. Mass spectra were obtained with the following conditions: glass capillary voltage, 4000 V; drying temperature, 200 °C; funnel 1 and 2 RF, 200 Vpp; hexapole RF, 100 Vpp; quadrupole ion energy, 10 eV; collision energy of 0 eV; and mass range, m/z 200 – 600 acquired with 5 Hz. Total sample time analysis was 5 seconds.

Results
In total, six different samples were analyzed and in all cases only the [M+H]+ ion was detected. The compounds LSD (m/z, 324.2058); 25C-NBOH (m/z, 322.1212); 25E-NBOH (m/z, 316.1917); 25I-NBOME (m/z, 428.0783); DOB (m/z, 276.0404); and 4’-fluoro-α-Pyrrolidinohexanophenone (m/z, 264.1768) were detected with a mass error of < 5 ppm. These compounds were analyzed by other techniques (GC-MS, IR and H-NMR) to confirm the results.

Discussion
The analytical technique proposed consists of a versatile tool for the direct and nondestructive analysis of diverse chemical compounds. Paper spray ionization is an easy-to-use ambient mass spectrometry technique, which is capable of desorbing/ionizing molecules from a sample surface under ambient conditions before its entering to the mass spectrometer. Generally, the combined use of solvent and high voltage provides an electric field on the paper tip leading to the formation of a spray as a Taylor cone, resulting in an ionization mechanism similar to an electrospray (ESI). The replacement of a high voltage source for a 9V battery simplifies the ionization source manufacturing, resulting in a less complex interface in comparison with standard ESI. However, in a zero voltage condition such as when battery is turned off, the analytical signal completely disappears. Despite these promising results, some limitations have been found in this method including low sensitivity at trace levels, low spray stability and ionization suppression in complex samples. In case of sensitivity, we hypothesize that it can be improved by the use of nanomaterial coated papers in order to enhance conductivity.

Conclusions
In this study, a low voltage paper spray ionization Q-TOF method for screening of NPS was developed. The analysis is relatively cheap, sensitive, easy and rapid, confirming its applicability and importance to forensic screening purpose. Besides its advantages, future improvements are still necessary to expand its application.
Abstract ID 138
Rapid detection of anabolic doping agents in non-invasive biological matrices using nanomaterial based SALDI-MS.
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Aim
Anabolic agents still account for the largest number of Adverse Analytical Findings (AAF) in recent testing figures from the World Anti-doping Agency (WADA). Detecting the array of anabolic doping agents that mimic the effect of testosterone in biological samples is uniquely complex compared to other performance enhancing drugs (PEDs), due in-part to an individual’s variation in endogenous testosterone. Testing for doping is further complicated by new designer substances continually being developed. Some of the difficulties in detecting doping and the conditions surrounding an AAF relate to how immediate and responsive testing can be within and around sporting events. The principle mass spectrometric analytical techniques used for the detection of doping agents can provide accurate and sensitive results but are limited in their ability to process samples on-site for immediate responsive testing. A focus has thus been placed on complementary techniques that have the capacity to be high-throughput and overcome these challenges.

Methods
The rapid analysis of a variety of doping agents in biological matrices was performed using DIOS/NIMS. This technique involves the use of nanostructured porous silicon substrates for low molecular weight detection. Through precise control of the nanostructured topography and surface chemical functionalisation, we were able to optimise surfaces for the detection of derivatized androgenic-anabolic steroids (AAS). DIOS surfaces detected AAS, including testosterone and androsterone. Using these surfaces we were also able to detect growth hormone releasing peptide-6, and stimulants, morphine and oxycodone, from biological fluids down to low ng/ml concentrations.

Results
A limit of detection (LOD) and limit of quantification (LOQ) was performed for several anabolic doping agents. This technique was further applied to various classes of substances currently present on the WADA Prohibited List, thus demonstrating its utility. Linear regression curves and limits of detection were performed, in water, for growth hormone-releasing peptide 6 (GHRP-6), cortisol, morphine, oxycodone, and testosterone. Determination of detection limits for morphine and oxycodone in saliva was performed, with limits of 1.11 and 1.62 ng/mL, respectively, and excellent linearity (R2>0.99) observed for each of the drugs. Analysis of derivatized AAS was performed for the sensitive detection of testosterone and androsterone, down to 0.18 and 0.77 ng/mL, respectively, from urine.

Discussion
The detection and optimization of doping agents and their metabolites was performed from spiked body fluids and compared against standard analytical methods. The determined limit of detection for a number of the assessed drugs was considerably lower than current WADA cut-off values. DIOS/NIMS was able to simultaneously detect these drugs in a single MS run. Analysis of all samples, in triplicate, was able to be performed on a single DIOS surface, thus reducing sample processing time.

Conclusions
Several validation experiments were performed demonstrating the capability of nanomaterial based SALDI-MS for the high throughput analysis of various classes of doping agents. Using a simple extraction procedure, analysis was able to be performed directly from biological matrices and results were obtained within minutes. This proof-of-principle study demonstrates high-throughput analysis for the drug testing of athletes using DIOS/NIMS, with the added flexibility to react and adapt to new low molecular weight drugs appearing on the market.
Abstract ID 178
Detection of synthetic peptides in dried blood spots by means of UHPLC-HRMS: a proof of concept for anti-doping analysis.

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Aim
Growth hormone releasing factors (GHRFs) are synthetic small peptides that stimulate the pituitary gland to release human growth hormone (hGH). These peptides are included in the World Anti-Doping Agency (WADA) list of prohibited substances because of their ability to enhance the natural level of hGH. Among GHRFs, Ipamorelin is a highly selective, potent and efficacious pentapeptide (Aib-His-D-2-Nal-D-Phe-Lys-NH2) which belongs to the class of GH secretagogues (GHS). As the current state of the art provides the detection of GHRFs in conventional matrixes such as urine, plasma or whole blood, the aim of the present study is to develop a method for the detection of Ipamorelin from dried blood spot cards (DBS) by means of ultrahigh performance liquid chromatography-high resolution/high accuracy mass spectrometry (UHPLC-HRMS).

Methods
Two drops of blood (previously spiked with Ipamorelin at different concentrations in the interval 5-50 ng/mL) were spotted on the Whatman® DMPK-C card and dried at room temperature to prepare DBS. A dedicated experimental design for the optimization of the extraction method varying incubation time and the solvent was performed. DBS were extracted by liquid extraction and, after ultrasonic treatment, the extracts were injected in an ExionLC UHPLC-system (Sciex) equipped with an Acquity CSH C18 column (100 x 2.1 mm, 1.7 μm particle size) protected by a C18 guard column. For the mass spectrometric detection, a X500R QTOF system (Sciex) was employed. The mass spectrometer was equipped with a Turbo V™ ion source (+ESI), and the acquisition was carried out following different MS experiments (full scan and MS/MS mode).

Results
The method was validated, with the main focus on qualitative result interpretation, considering the parameters specificity, linearity, recovery, limit of detection, precision, and ion suppression/enhancement effects. The ipamorelin structure was confirmed acquiring high-resolution fragmentation spectra. The mass error of the precursor and fragment ions was lower than 5 ppm. Ipamorelin was successfully detected in DBS with a detection limit of 5 ng/mL.

Discussion
Despite the DBS analysis requires more efforts, there are a lot of advantages in terms of sampling, storage and speed making DBS increasingly used in different fields. Particularly, in the anti-doping field, the use of DBS matrix could reduce the costs of sample collection and management of samples, increasing the number of samples to be tested. Although the use of DBS sampling is not new, it is mainly applied for the detection of small molecules, and only recently there is an interest in peptides and proteins as target analytes for DBS analysis.

Conclusions
In this study, we demonstrated that DBS could be a suitable sample matrix for the detection of small peptides in blood, in particular Ipamorelin, at low levels. Further studies are currently underway to increase the method sensitivity, expand the panel of target analytes and apply the proposed method to real samples.
Abstract ID 262
Optimization of a method for the detection of anabolic androgenic steroids in dried urine spots by paper spray-mass spectrometry ionization.

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Aim
The abuse of non-therapeutic anabolic androgenic steroids (AAS) has been growing rapidly among athletes and non-athletes. The misuse is considered a health problem of considerable importance, since AAS are the principal drugs found in forensic doping cases. Therefore, toxicological analyses are the safest approach to identify the abuse of AAS in sports according to WADA’s World Anti-Doping Code, detecting the use of these substances by athletes in order to achieve competitive advantage. This work aims to optimize a method for the identification of AAS and its main metabolites in dried urine spots (DUS) by paper spray-mass spectrometry ionization (PS-MS).

Methods
Aliquots of 3mL of urine samples were fortified with 20µL of methyltestosterone (internal standard-IS) at the concentration of 200ng/mL and 0.75 mL of phosphate buffer pH 6.8. 50 µL of E. coli K12 β-glucuronidase enzyme solution was added and the sample was incubated at 50 °C for 60 min. The analytes tested were 19-norethicholanolone, 19-norandrosterone, 3-hydroxystanozolol, 16-beta-hydroxystanozolol, epitestosterone, 17-alpha-methyl-5-beta-androstan-3-alpha-17-beta-diol, 17-alpha-methyl-5-alpha-androstan-3-alpha-17-beta-diol, trembolone, testosterone and androstenedione. To identify the best paper type for AAS analysis, optimization assays were performed on Whatman 903 Protein Saver Card and qualitative filter paper. Both papers were cut in triangular format in 5x10mm with an angle of 30°C. An aliquot of 10µL of the AAS pool was added on the filter paper and the sample dried for 2h. The paper was allocated at 5 mm from the MS inlet. Methanol (20µL) was added to the paper for data acquisition. Eleven solvents (methanol, methanol: water 1:1, methanol:water 90:10, methanol:formic acid 0.1%, acetone, acetonitrile, acetonitrile: water, acetonitrile: water 1:1, isopropanol, isopropanol: water 75:25) were tested for the optimization of the spray solvent. The assays were performed at the concentrations of 10, 75 and 200 ng/mL in a pool of AAS in the urine. An aliquot of 10 µL of the AAS pool was added to the filter paper and the sample was dried for 2h at room temperature. For this analysis, 20 µL of each solvent was added to the paper. In order to test the addition of the IS to obtain better signal intensity, triplicate samples were added with 20 µL of IS in 500 µL of urine at a concentration of 200 ng/mL and addition of 10 µL of IS in methanol at a concentration of 200 ng/mL directly on paper. The paper was dried for 20 min at room temperature. All analyses were performed on the LCMS 8030 triple quadrupole mass spectrometer with electrospray ionization (Shimadzu Corporation, Kyoto, Japan). LabSolutionsTM software was used for data acquisition and processing.

Results
Whatman 903 Protein Saver Card paper showed lower absolute intensities of the ion fragments and the chronograms. However, the qualitative filter paper produced the highest peak intensities of the studied analytes. Thus, the qualitative filter paper was selected for the analyzes of this study. The optimum solvent was analyzed for each analyte according to the absolute intensity of the fragments and the chronograms formed, and acetonitrile showed better extraction. The impregnation of the IS on filter paper had higher absolute intensities than the addition of the same amount in the urine sample, with subsequent application on the paper.

Discussion
Whatman 903 Protein Saver Card is described in the literature for analysis in DUS but it is more absorbent than the filter paper, so it requires a greater amount of sample. In comparison, papers with smaller grammage require less amount of sample and solvent, allowing better analyzes in PS-MS. The optimum solvent was analysed for each analyte according to the absolute intensity of the fragments and the schedules formed. It is important that the solvent used is capable to increase the sensitivity and selectivity of the method, allowing the molecules to be extracted while the urine components remain retained. The addition of the internal standard was tested in order to obtain better signal intensity. The impregnation of the IS in the filter paper is interesting for the cases where the DUS sample is sent to the laboratory and it is not possible to add the IS in the biological matrix in the liquid phase. In this way, the IS could be previously added to the paper.

Conclusions
This study intended to optimize a method for the detection of AAS in dried urine spots by paper spray-mass spectrometry ionization. This ambient ionization technique is an innovative approach for the analysis of AAS because it reduces sample volume to 10 µL, costs, solvent, storage and supplies. It also decreases data acquisition time to 1 minute with minor sample handling, minimizing the biological risk to the analyst. The results meets with WADA requirements regarding the method sensitivity for the analytes detection (2-10 ng/mL). This study was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - AUXPE 3419/2014.
Abstract ID 307
Mass spectrometric characterization of Melanotan II, a skin pigmentation enhancer sold on the black market of PIEDs.
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Aim
Forensic toxicology laboratories are often required to identify unknown compounds, such as Novel Psychoactive Substances (NPS) and Performance and Image Enhancing Drugs (PIEDs), in both seized materials and biological fluids, sometimes very quickly and often without the availability of reference standards or analytical data from scientific literature.

Indeed, PIEDs are often illegally sold on the internet, which acts as an ideal platform to promote and market these substances. Among these products, Melanotan II, a synthetic analogue of α-melanocyte stimulating hormone, is designed to induce the production of melanin and enhance sunless skin pigmentation. In addition, it is used as a sexual stimulant. The misuse of Melanotan II, sometimes referred to as the “Barbie drug,” can be related to acute adverse reaction, like facial flushing, nausea, and vomiting, as well as cutaneous diseases, such as non-homogeneous skin pigmentation, hematomas, growth/darkening in existing nevi or newly emerging nevi, which carry a high risk of developing into melanoma.

In this study we characterized, in the absence of a reference analytical standard, the PIED Melanotan II, found in three unlabelled bottles seized by police together with anabolic agents, by liquid chromatography – high-resolution accurate-mass OrbitrapTM mass spectrometry (LC-HRMS). To the best of our knowledge, no applications of LC-HRMS which includes the study of the ion fragments by accurate mass measurement, have been proposed so far for Melanotan II identification.

Methods
Seized materials, consisting in a flocculent white powder, contained in unmarked glass vial, sealed by an aluminium cap with a yellow plastic top, were weighted and dissolved in methanol. The solutions obtained were firstly injected in GC-MS and subsequently in LC-HRMS; both the acquisition modes were in full-scan. The analysis for the latter instrumentation was performed initially at 100,000 full width at half maximum resolution (FWHM) without fragmentation, to elucidate the elemental compositions of unknown molecules. A further injection applying in source 40 eV fragmentation voltage was used in order to study the accurate masses of the obtained characteristic fragments.

Results
GC-MS allowed the identification of tripyrrolidinophosphine oxide, recognized by comparison with NIST and Wiley spectral libraries. LC-HRMS analysis showed the presence of a peak which corresponded to single charge ions at m/z 1024.5479 and double charge ions at m/z 512.7775. Accurate mass measurement of both ions had mass accuracies of 0.39 and 0.20 ppm, respectively. The isotopic pattern of both ions were consistent with the theoretical exact mass of a compound with elemental formula C50H69N15O9. Moreover, a relative isotopic abundance (RIA) value of 12% and 14%, for single charge and double charge ions respectively, were obtained. Furthermore, accurate mass measurement of the characteristic MH+ collision-induced product ions obtained from LC-HRMS analyses enabled to obtain the structural characterization of Melanotan II. Also, LC-HRMS analysis confirmed the presence of tripyrrolidinophosphine oxide, probably used to initialize the crystallization at the end of the synthesis process.

Discussion
Although GC-MS and LC-HRMS generally give complementary information for compound identification, in this case only LC-HRMS gave valuable information for the identification of this oligopeptide compound, due to the non-volatile characteristics of the compound under study.

The application of LC-HRMS allowed us to obtain accurate mass measurement of Melanotan II single and double charge quasi-molecular ions, in accordance with the elemental formula C50H69N15O9. The experimental and calculated isotopic patterns of both ionic species and the obtained RIA value confirmed the assignment of the above elemental formula. Accurate mass measurement of the characteristic MH+ collision-induced product ions obtained from LC-HRMS analyses enabled to obtain the structural characterization of Melanotan II.

Conclusions
The use of LC-HRMS allowed the identification, without using reference standards, of the synthetic peptide Melanotan II, and of the by-product tripyrrolidinophosphine oxide, in the unlabeled products seized by police. LC-HRMS confirmed to be a powerful analytical technique to elucidate the elemental composition and structural characteristics of unknown drugs present in seized materials and often in biological samples.
Abstract ID 401
Undisputable results by coupling of GC-IRMS with high-resolution mass spectrometry for final confirmation in sports drug testing.

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Thermo Fisher Scientific

Aim
Endogenous steroids are the most frequently detected class of substances misused in sports.

In doping control, gas chromatography (GC) coupled with isotope ratio mass spectrometry (IRMS) is now routinely applied. It allows distinguishing between endogenous steroids from their synthetic analogs in urine by the determination of 13C/12C isotope ratios.

Methods
With the introduction of the Thermo Scientific™ GC Isolink II™ conversion unit, the IRMS system can be coupled with any organic MS system connected to a single Trace 1310 GC. Concomitant data are critical to qualify the true identity of a compound. This has become mandatory in confirmation of so-called adverse analytical findings. From a single injection, the structure and isotope ratio of each compound can be determined.

Results
Here we present the results of an excretion study after testosterone administration. One male volunteer was administered 30 mg of testosterone orally. Urine samples collected were prepared according to the standard protocol of the doping control routine and analyzed by a GC-IRMS system online coupled with a Q Exactive GC. The Q Exactive GC Orbitrap™ GC-MS/MS is a new class of GC-MS system with high mass resolution and exceptional mass accuracy for the detection and identification of potentially co-eluting matrix compounds. By coupling GC-IRMS with the Q Exactive GC system, the isotopic compositions and the comprehensive qualitative and quantitative sample information with high levels of selectivity, sensitivity, and confidence are accessible simultaneously.

Discussion

Conclusions
The data demonstrates that testosterone doping can be still approved after 24 hours by utilizing the IRMS results of testosterone and its main metabolites. The administration of 30 mg of testosterone for the purposes of this study can be considered to be a low and realistic dose typically applied in sports.
Abstract ID 454
Analysis of seizures formulations of stanozolol in South Brazil by High Resolution Mass Spectrometry.
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Aim
The anabolic-androgenic steroids (AAS) are synthetic derivatives of testosterone and is used medically for several diseases. However, misuse are commonly observed by professional and unprofessional athletes to promote the increase strength, bodyweight and performance. Black market AAS are frequently obtained by internet from clandestine drug manufacturing laboratories, without any quality standards, being potentially dangerous for users. The purpose of this work was the development and application of a fast and simple procedure for the quantitation of stanozolol by liquid chromatography high-resolution mass spectrometry (LC-HRMS) in samples seized in Rio Grande do Sul state.

Methods
Sample procedure: The stanozolol samples were separated by pharmaceutical forms. Aliquots of the samples were added with internal standard, dissolved in methanol and 5μL were injected into the analytical system. Instrumentation: A micrOTOF-Q III (Bruker Daltonics, Bremen, Germany) coupled to a Shimadzu Prominence liquid chromatography system (Shimadzu, Kyoto, Japan) was used for the analysis. Mass spectra were obtained with the following conditions: capillary voltage, 3000 V; nebulizer gas, 3 bar; drying gas, 5 l/min; drying temperature, 150 °C; quadrupole ion energy, 4 eV; mass range, m/z 50 - 350 acquired with 1 Hz. The following chromatographic condition was used: a 75 × 2.0 mm i.d., 2.2 μm, Shim-pack XR-ODS II (Shimadzu, Kyoto, Japan) eluted with a gradient of 0.1% formic acid in water (A) and acetonitrile (B) at a 400 μL/min flow rate and 45°C as follows: 0 – 2.5 min, 40 – 100% of B; 2.5 – 3.5 min, 100% of B; 3.5 – 3.6 min, 100 – 40% of B; 3.6 – 5 min, 40% of B.

Results
The method was validated according to national and international guidelines. All parameters evaluated in the method were considered satisfactory. The newly developed method has been validated for limits, intra-day and inter-day precision, accuracy, selectivity and matrix effects. The linear weighted regression models were used. The lower limit of quantification (LLOQ) was 0.1 µg/mL. The precision study was performed with analysis of six replicates on each of three days, and the precision data (within and between-day) were calculated using one-way ANOVA with day as a grouping variable to adequately account and combine for within and between day effects. The results were expressed as percent relative standard deviation (%RSD). The results obtained of intra-day precision presented a variation of 11.4 - 17.8%. For inter-day precision the controls values were encountered in the range of 11.8 to 16.7%.

Discussion
The developed method was successfully applied to 31 samples seized by Secretariat of the Federal Revenue of Brazil. It was observed that 90% of the medicines were adulterated and of this percentage, 65% have higher concentrations than those indicated on the package.

Conclusions
This work established a new method of quantification of stanozolol in LC-HRMS with success. This method aims to contribute to the analysis of the identification and quantification of the anabolic androgenic steroid frequently seized by federal inspection agencies.
Abstract ID 506
The working horse Q-exactive Focus, 2 years of screening doping control samples.

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Doping Control Laboratory

Aim
In order to increase the sample capacity, the Stockholm Doping Control Laboratory set up a LC-HRMS screening method including about 450 substances in different substance classes (diuretics, narcotics, anabolic steroids, etc.) using LC-HRMS.

Methods
Human urine doping control samples (2 mL) are analyzed on a Q-exactive Focus (Thermo) after a combined hydrolysis, SPE clean-up and dilute-and-shoot sample preparation. The hydrolysis is performed using β-glucuronidase from E.coli and the SPE is a strong cation exchanger from Chromabond (60 mg, 3 mL). The SPE cleanup includes 3 washing steps: Milli-Q H2O, 2 % HAc(aq) and 10 % MeOH. The elution is done with 1.25 % ammonia in MeOH/ACN. The analysis is performed in fullscan mode with a PRM inclusion list. Mobile phase A and B both contain 10 mM ammonium format buffer (pH 4.5), and MilliQ-H2O for A and 90 % MeOH(aq) for B. The gradient is linear, starting at 2 % B up to 90 % B.

Results
The method is validated and fulfills the criteria in TD2018MRPL and includes several substance classes with differences in both chemical properties, ionization polarities and detection limit, ranging from nanograms to micrograms per milliliter.

Discussion
The Q-exactive Focus is a basic Q-exactive without some features. For example, max resolution (FWHM) is 70 k instead of 140 k and the mass range is 50 to 3000 m/z instead of 50 to 6000 m/z. These adjustments to the features of the Q-exactive reduce the price of the instrument and, depending on the goal, type of the analysis, and the amount of money possible to spend on an instrument, the Q-exactive Focus can be the instrument of choice (fit-for-purpose). Also, since the sample hotel is kept cold (+ 5°C), it gives a good storage environment for the samples in case re-injections are needed.

Conclusions
By coupling the Q-exactive Focus to a CTC injector with a sample hotel, high capacity was achieved, and the instrument has performed well in our anti-doping control screening for the last two years. The screening for doping substances fulfills the criteria of minimum required performance levels stated in the technical document TD2018MRPL from World Anti-Doping Agency (WADA).
Abstract ID 15
High-throughput determination of tegafur and 5-fluorouracil in human tear and plasma by HILIC-MS/MS.

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Aim
TS-1® is composed of tegafur (FT), gimeracil, oteracil (potassium and is widely used as an antitumor drug. FT is a prodrug and is metabolized to pharmacologically active 5-fluorouracil (5-FU) in the liver. Severe canicular stenosis and nasolacrimal duct blockage have been reported as side effects of TS-1 therapy. A new high-throughput method was developed to analyze FT and 5-FU in tears and plasma by hydrophilic interaction liquid chromatography (HILIC)-tandem mass spectrometry (MS/MS).

Methods
The ultra-fast liquid chromatography system (Shimadzu Corp., Kyoto, Japan) and a hybrid API 4000 QTrap mass spectrometer (Sciex, Framingham, MA, USA) were used in the present method. Tear (10 µL) spiked with FT, 5-FU and 5-chlorouracil (IS) was diluted with 40 µL of 2 M ammonium acetate and 250 µL of acetonitrile with 2% formic acid; and 20 µL of plasma spiked with two drugs and IS was diluted with 80 µL of 2 M ammonium acetate and 500 µL of acetonitrile with 2% formic acid. After centrifugation (19,600 × g for 3 min), the clear supernatant extract (15 µL) was directly injected into the HILIC-MS/MS, and each drug was separated on a Unison UK-Amino column (50 mm × 3 mm i.d., particle size 3 µm) with a linear gradient elution system composed of a 10 mM ammonium acetate solution (pH 6.8, solvent A) and acetonitrile (solvent B) at a flow rate of 0.7 mL/min. The gradient runs were programmed to change from 10% solvent A/90% solvent B to 12% solvent A/88% solvent B within 1.0 min. Quantification was performed by multiple reaction monitoring (MRM) with negative-ion atmospheric pressure chemical ionization (APCI). The MS/MS (precursor of the product) transitions used for this HILIC-MS/MS analysis were m/z 199→42 for FT, m/z 129→42 for 5-FU, and m/z 146→42 for IS.

Results
Distinct peaks appeared for the drugs on each MRM channel within 2 min. The regression equations showed good linearity within the ranges of 0.04–4.0 µg/mL for tear and plasma, with the limits of detection at 0.02–0.04 µg/mL for both samples, respectively. Recoveries for FT and 5-FU for tear and plasma were 99–128% and 95–102%, respectively. Intra- and inter-day coefficients of variations for two drugs were less than 10.8%. The accuracies of quantitation were 97-115% for both samples. In our study, for the quantitative evaluation of the matrix effect, we analyzed artificial tear solution (ATS) and five individual blank plasma samples from healthy volunteers. The percentage matrix suppression effect were -2.6 to 13.4. However, the matrix effect did not cause quantification bias, as evidenced by the CV values of 2.7–7.4%. Our method was successfully applied to the actual analyses of real patients who were administered the TS-1® medication.

Discussion
The UK-Amino column has an aliphatic amionopropyl group bonded to a silica surface and the pKa of this primary amine functional group is around 9.8. In the present study, we identified good HILIC separation of the FT and 5-FU on the UK-Amino column using a mobile phase comprising of 10% of 10 mM ammonium acetate (pH 6.8)/90% of acetonitrile to 12% of 10 mM ammonium acetate (pH 6.8)/88% of acetonitrile by linear gradient elution. Moreover, to maximize the APCI-MS sensitivity, a flow rate of 0.7 mL/min was selected. This provided a short analysis time of 2 min and a relatively low back-pressure of ~4.3 MPa with the initial mobile phase. Furthermore, the presence of a Waters in-line filter as a guard column for the HILIC-MS/MS system resulted in the UK-Amino column having excellent resistance against degradation and a column that could be used repeatedly, for at least 300 injections, with good reproducibility. The method was successfully applied to determination of the levels of FT and 5-FU in tear and plasma samples after administration of TS-1®.

Conclusions
This method was applied to real patient tear and plasma samples to confirm its effectiveness. Patient A was a 65-year-old man (body weight = 49 kg) with hypopharyngeal cancer and treated with TS-1®. After a one-week washout period, 60 mg of TS-1® was administered as a single dose through a gastric fistula. Patient B was a 79-year-old woman (body weight = 47 kg) with gastric cancer and treated with TS-1®. After a one-week washout period, 120 mg of TS-1® was administered as a single dose. The tear and whole-blood samples were drawn before intake and 4 or 5 h after administration. The tear samples (10–15 µL) were carefully collected using Microcaps® Disposable Micropipettes (Drummond Scientific Co., Broomall, PA, USA). The whole-blood samples (5 mL) were collected.
Abstract ID 28
Prediction of Synthetic Cannabinoids LC Retention by Quantitative Structure-Property Relationship as an aid to identification of new/unknown compounds.

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Aim
Despite the indisputable power of algorithms based on MS information in the identification of new/unknown compounds, predicting chromatographic retention times (RT) provides a significant aid to discriminate among structural isomers. Moreover, predicted RT information can be easily implemented in automated search routines with no need for a reference library, whereas predicted MS and MS2 fragmentation is less prone to automation. Chromatographic Retention time (RT) may be predicted from the chemical structure of a solute using Quantitative Structure-Property Relationship (QSPR) analysis. The aim of this work was to construct a QSPR model for the prediction of reversed-phase liquid chromatography RT of synthetic cannabinoids (SC) using theoretical molecular descriptors and fingerprints.

Methods
The absolute and relative (internal standards: JWH-018-d9, JWH-018 N-pentanoic acid metabolite-d5, JWH-073 N-(4-hydroxybutyl) metabolite-d5) RT of 102 SC (80 parent compounds and 22 metabolites) were measured with an Agilent LC/Q-TOF MS system (Infinity 1290 LC; 6550 iFunnel Q-TOF MS). Separation was performed on an Agilent ZORBAX Eclipse Plus C18 (100 x 2.1 mm, 1.8 µm) equipped with a Phenomenex SecurityGuard cartridge, in gradient mode (A: 5 mM ammonium formate buffer, pH 4; B: acetonitrile with 0.1% v/v formic acid; 10% B for 0.5 min, linear increase to 90% B in 9.5 min, 1 min at 90% B, step increase at 100% B, 0.29 min at 100% B, then back to initial conditions and 1.2 min equilibration; flow rate, 0.5 ml/min; column temperature, 50°C). SMILES (Simplified Molecular-Input Line-Entry System) notations of the 102 training compounds obtained from different sources (PubChem Compound, Chemspider, Cayman Chemical), or generated from the structure with ACD/ChemSketch (Ver. 2017.1.2), were converted into canonical SMILES and then into MDL (Molecular Design Limited).mol format using OpenBabel software (Ver. 2.4.1; http://openbabel.org/wiki). The latter format was used to calculate over 5600 molecular descriptors and fingerprints with PaDEL-Descriptor (Ver. 2.21; http://www.yapcwsoft.com/dd/padeldescriptor/). After removing collinear (multiple correlation coefficient <0.95) and low variance (minimum standard deviation, 0.01) descriptors by means of Unsupervised Forward Selection (http://www.vcclab.org), the QSPR multiple linear regression model for relative RT (rRT) prediction was constructed using stepwise regression with R (Version 3.5.2).

Results
The final model, that included 56 molecular descriptors/fingerprints, had an adjusted R2 of 0.9735. The model was submitted to Leave-One-Out cross validation (Root Mean Squared Error, RMSE = 0.1064; R2 = 0.7601) and to 10-fold cross validation (RMSE=0.1015; R2=0.7869). Average 95% confidence interval (CI) of prediction of rRT was ±0.1 (RT±0.65 min).

Discussion
The model proved to perform well in predicting RRT of parent compounds, as well as of metabolites of the less recent SC, whereas performance worsened (95% CI of rRT, ± ca. 0.15) for metabolites of the last generation of SC, not included among the training compounds.

Conclusions
While accurate mass measurement allows to identify the molecular formula of unknown compounds, predicting retention time with reasonable precision enables to discriminate among structural isomers, thus providing a significant aid in reducing the list of candidates and in discriminating between structural isomers.
Abstract ID 35
Parental exposure to recreational drugs and alcohol in neonates admitted to a neonatal intensive care unit.
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Aim
To investigate the incidence of exposure to recreational drugs and alcohol in neonates admitted in neonatal intensive care unit (NICU) by biomarkers analysis in neonatal biological matrices. To investigate if exposed newborns present significant differences in anthropometric characteristics and outcomes when compared to not-exposed ones and identify maternal profiles associated with the consumption substances.

Methods
Prospective observational study carried out in Palma de Mallorca Hospital over a 12-month period. Population: 1) Newborns admitted to NICU without suspicion of prenatal exposure to recreational drugs and/or alcohol, whose parents wrote an informed consent. 2) All neonates admitted to NICU with clinical suspicion of prenatal exposure. Clinical criteria for suspicion: In the newborn: hypertonia, irritability, exaggerated crying, tremors, tendon reflexes, unjustified restriction of intrauterine growth; in the mother: history or suspected abuse of alcohol and/or drugs; uncontrolled pregnancy or poor control; and/or teen mothers. Methods: Neonatal meconium was collected immediately after birth. A DRI® assay was used to screen meconium specimens for seven drug classes and positive results were confirmed by gas chromatography-mass spectrometry. Ethyl glucuronide (EtG) in hair and meconium was measured by ultra-performance liquid chromatography–tandem mass spectrometry (cut-off of 30 ng/g meconium and 7 pg/g hair). Toxicological results were compared with data obtained from: structured in-person baseline maternal interview and medical records. Statistical analyses: Comparisons between groups were performed using one-way ANOVA and independent-samples t-test or Kruskal-Wallis and Mann–Whitney U test for continuous variables, whereas chi-square or Fisher’s exact test was applied for categorical variables. A p value <0.05 was considered statistically significant.

Results
From a total of 488 newborns admitted in the NICU, only 220 cases fulfilled the inclusion criteria were enrolled in the study: 28 (12.7%) cases with clinical suspicion of exposure and 192 (87.3%) without suspicion. Thirty-two (14.6%) cases had positive toxicology: 11 (5.7%) were newborns without clinical suspicion and 21 (75.0%) with clinical suspicion. Cannabis was the most frequent detected substance (n=19, 8.6%), EtG (n=15, 5.9%), cocaine (n=8, 3.6%) and opiates (n=1, 0.5%) were also detected. EtG concentrations ranged from: 56.4 (quartile 1)-345.2 (quartile 3) ng/g meconium (median 80.3 ng/g) and eleven newborns (5.0%) presented EtG concentrations under the cut-off. A single substance was positive in 25 cases (78.1%): 13 (52.0%), 9 (36.0%) and 3 (12.0%) times for cannabis, EtG and cocaine, respectively. Poly-drug use was detected in 7 (21.8%) cases.

Newborns exposed to any recreational drug or alcohol resulted to have differences with respect to non-exposed ones in the following variables: lower maternal age (30.0 vs. 33.4 years), adequate prenatal care (71.9 vs. 99.5%), percentile length at birth (31.1 vs. 42.6), prematurity (21.1 vs. 66.0%), estimated gestational age at birth between 28-34 (21.9 vs. 43.1%), use of surfactant (0 vs. 12%), enteral nutrition (32.3 vs. 59.3%) and duration of hospital stay (16.5 vs. 22.5 days); and higher suspected maternal recreational drug abuse (31.3 vs. 0%), self-reported drug, tobacco and/or alcohol use (38.8 vs. 5.9%), APGAR score 1 minute (7.7 vs. 7.0), estimated gestational age at birth (256 vs. 241 days), and estimated gestational age at birth >34 weeks (78.0 vs. 53.2%). Predictive factors of fetal exposure: in case of cannabis: younger age of the mother, suspected maternal recreational drug abuse, inadequate prenatal care and self-reported consumption of cannabis and tobacco. In case of EtG: uncontrolled pregnancy and self-reported consumption. In case of cocaine: uncontrolled pregnancy, suspected maternal recreational drug abuse, inadequate prenatal care and self-reported consumption.

Detected substances in newborns without clinical suspicion were: EtG (n=8, 72.7%), cannabis (n=4, 36.4%) and cocaine (n=1, 9.1%). No mother recognized consumption. Detected substances in newborns with clinical criteria of drug exposure were: cannabis (n=15; 71.4%), cocaine (n=7, 33.3%), EtG (n=5, 21.8%) and opiates (n=1, 4.8%). Only half of the mothers recognized consumption.

Discussion
The estimated percentage of exposure to recreational drugs and alcohol in newborns admitted to the authors NICU was 9.6% being 5.7% in newborns without clinical suspicion and 75% with clinical suspicion. Newborns exposed to substances of abuse presented worse results in the analyzed variables. Cannabis use can be associated with younger age of mothers, while uncontrolled pregnancies were associated with the consumption of alcohol or cocaine. There is a risk of underestimating newborns exposed to drugs or alcohol if only clinical criteria or mother self-report is used. Two limitations of our study were that the biomarkers used give no information on maternal substance misuse in the earlier stages of pregnancy and tobacco smoking only was registered in maternal interview.

Conclusions
Objective assessment of prenatal exposure to recreational drugs and alcohol provides the basis for appropriate baby treatment and adequate medical and social follow-up. Neonatal treatment was affected by the results of the biomarker analyses. In positive cases, breastfeeding was withdrawn if the mother’s consumption continued, abstinence scores were performed and complementary tests were requested to the newborns. In addition, social intervention and family follow-up were carried out.
Abstract ID 58
Sensitive liquid chromatography/tandem mass spectrometry method for the simultaneous determination of thirteen beta blockers.

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Aim
In recent years, examples of drugs responsible for acute drug intoxication in elderly people include benzodiazepines, phenobarbital, digitalis preparations, phenytoin, beta blockers or calcium antagonists, and antidepressants in Japan. Due to the diversity of underlying diseases, the reasons for this include increasing number of prescription medicines due to regular oral medicines themselves interacting, and age-related changes in drug metabolism. Beta blockers for clinical use are employed in the management of a range of disorders, including hypertension, heart failure, arrhythmias, migraine headache and tremor. Cardiovascular drugs (beta blockers) present more serious symptoms when misused than the underlying diseases themselves. Because there are many types of beta blockers and are widely prescribed, they are likely to be misused. A simultaneous analysis method of thirteen beta blockers including bisoprolol and carvedilol, which are frequently used clinically in Japan, have not been reported yet. The aim of this study is to develop and validate a sensitive liquid chromatography–tandem mass spectrometry (LC-MS/MS) with ESI method for the simultaneous determination of thirteen beta blockers (atenolol, carteolol, nadolol, pindolol, timolol, acebutolol, arotinolol, metoprolol, labetalol, bisoprolol, propranolol, betaxolol, carvedilol).

Methods
The extractions of beta blockers in human serum were performed by solid-phase extraction method using Oasis® PRIME HLB column (Waters). LC-MS/MS experiments were performed with a HPLC system, which consisted of Shimadzu LC-20AD pumps, a SIL-20AC autosampler and the 4000 QTRAP mass spectrometer (AB SCIEX). The chromatographic separation was performed on a Mightysil-RP-18 MS column (150 x 2.0-mm i.d., 5 µm). For the gradient elution, two solvents were used: (A) 10 mM acetic ammonium buffer and (B) acetonitrile. The flow rate was 0.20 mL/min and the column and autosampler were maintained at 37 and 4°C, respectively. The settings of the turbo ion-spray were as follows; curtain gas, N2:40 psi, collision gas, N2:4 psi, collision energy: 27–41V, ion-spray voltage: 5500 V, ionization mode: ESI positive, source temperature: 600°C.

Results
Separation and sensitivity for the detection of thirteen beta blockers by LC-MS/MS were sufficient and the precursor [M+H]+ ion was detected in the mass spectrum for each drug. This method had a chromatographic total run time of 15 min. The calibration curves were linear over the concentration range of 20-400 ng/mL for thirteen beta blockers (r=0.9709 ~ 0.9997).

The extraction yields of human serum sample with Oasis® PRIME HLB column were ranged from 66.2 to 93.5%, the precision within 7% CV values were acceptable of this method. But the extraction yields of carvedilol was low(26.2%). Currently, we are experimenting to improve the extraction yields of carvedilol.

Discussion
Phyo Lwin EM, et al (2017,Bioanalysis.) developed a method for the determination of atenolol in human plasma and milk sample by LC-MS/MS. The limits of detection (LOD) and quantification (LOQ) were in the range 1.0 ng/mL and 5.0 ng/mL for atenolol. Our LC-MS/MS with ESI method was able to measure compounds at approximately the same concentrations as the analytical methods of Phyo Lwin EM, et al. In our experiments, LOD and LOQ in the range 0.0034-0.14 ng/mL and were 20 ng/mL for thirteen beta blockers. Using this simultaneous analysis method, the LOQ were approximately the same as therapeutic blood concentration at which thirteen beta blockers are clinically used.

Conclusions
The presently established method is very useful for simultaneous measurements of beta blockers in human serum by LC-MS/MS in clinical and forensic investigations.
Abstract ID  69
Benzodiazepines facilitate intoxication to oxycodone: evidence of metabolic interaction with diazepam and a designer drug, diclazepam
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Aim
Little is known about potential metabolic interactions between opioids, benzodiazepines (BZD) or designer benzodiazepines (DBZD). However, only a slight modification in the structure of a BZD will have a high impact on its pharmacokinetic profile and can also lead to important interactions in chronic or acute intoxications.

Our aim was to explore metabolic interactions between oxycodone (OXC), one of the most prescribed and abused opioids, and diazepam (DZ) or, its derivative, diclazepam (DCZ), one of the most popular DBZD. Acute and chronic patterns were taken into consideration.

Methods
In a first step, we performed in vitro experiments (liver microsomes): knowing that noroxycodone and oxymorphone are respectively the main and the minor metabolites of OXC, we explored the evolution of noroxycodone/OXC and oxymorphone/OXC ratios when adding increasing concentrations of DCZ (5 and 10 μg/ml) or DZ (5 and 10 μg/ml).

In a second approach, we performed in vivo experiments with Swiss mice. (i) To explore acute toxicity, concomitant single doses of OXC (1.5 mg/kg) with DZ (5.0 mg/kg) or with DCZ (0.5 mg/kg) were administered to mice. Blood concentrations of the drugs and their metabolites (nordazepam, temazepam, oxazepam for DZ; lorazepam, lorazepam for DCZ; plus metabolites of OXC) were measured at 0.5, 1.5 and 3h. (ii) To mimic chronic intoxication with a DBZD, DCZ was introduced on day 10 to mice continuously infused with OXC. Drugs and metabolites were measured in blood and urine on day 15. For each protocol, motor coordination and pain tests were performed. Measurements in liver microsomes preparations, blood and urine samples were performed using LC-MS/MS methods.

Results
In the in vitro experiments, the addition of DZ or DCZ at 5 and 10 μg/ml significantly decreased oxymorphone/OXC ratio. We observed OXC only: 0.26 ± 0.02%; OXC + 5 μg/ml DZ: 0.14 ± 0.02%; OXC + 10 μg/ml DZ: 0.15 ± 0.01%; OXC + 5 μg/ml DCZ: 0.15 ± 0.01%; OXC + 10 μg/ml DCZ: 0.12 ± 0.01% (p<0.05, ANOVA). Noroxycodone/OXC ratio decreased significantly only in the presence of 10 μg/ml of DCZ (OXC only: 1.94 ± 0.22 versus OXC + 10 μg/ml DCZ: 1.10 ± 0.03% (p<0.05, ANOVA).

For acute toxicity in mice, we observed that DZ and DCZ were able to inhibit OXC metabolism: Noroxycodone/OXC ratio significantly decreased from 19.29 ± 8.02 for OXC alone vs 11.07 ± 7.14 for OXC + DZ and to 3.70 ± 1.80 for OXC + DCZ (p<0.05, ANOVA). On the contrary, OXC did not impact DZ and DCZ metabolism. In the chronic condition, we observed that DCZ enhanced the accumulation of oxymorphone: oxymorphone/OXC ratio in the presence of DCZ = 0.13 ± 0.04 while oxymorphone was undetectable in the absence of DCZ. On the contrary, the addition of DCZ did not modify noroxycodone/OXC ratio.

Discussion
Death by overdose and polydrug abuse frequently involve opioids and BZD. Efforts made to deal with this public health problem are partially inefficient due the increased use of DBZD which are commonly co-abused with opioids. In the present study we explored 2 common situations: (i) the consumption of OXC with a commercial BZD or (ii) with an illicit BZD. Using two in vitro and in vivo approaches, we demonstrated that DZ and DCZ interact with the metabolism pathways of OXC. An acute dose of these BZD increases the accumulation of OXC. In chronic condition, DCZ promotes the production of oxymorphone, which is the most potent and toxic metabolite.

Conclusions
These results support the fact that overdoses to OXC are favoured in the presence of DZ and DCZ. Other metabolic interactions should exist between this opioid and other BZD.
Abstract 

Stability of Amphetamines in Dried Blood Spots by using GC Methods.

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Aim

Recently, analysis of dried blood spots (DBS) is an increasingly accepted method in therapeutic drug monitoring and forensic toxicology. Consequently, stability studies of validated Gas Chromatography Mass Spectrometry (GC-MS) method for amphetamine, methamphetamine (MA), methylendioxyamphetamine (MDA), methylendioxymethamphetamine (MDMA), and methylendioxyethylamphetamine (MDEA) in DBS are required. The objectives of this study are to evaluation a (GC-MS) method to determine amphetamine and its derivatives in dried blood spots on filter paper and to investigation the stability of amphetamine drugs in the blood spots using GC-MS method during storage for 5 months at three different temperature degrees (room temperature °C, 4°C, and -20°C).

Methods

Twenty-one samples were analyzed for all Amphetamine and its Derivatives. Sample preparation was included liquid–liquid extraction with ethyl acetate in the presence of sodium hydroxide as alkaline medium. Methamphetamine-D5 (MA-D5) and methylendioxyamphetamine-D5 (MDA-D5) were used as internal standards (IS). The sensitivity was evaluated by determining limits of detection (LOD) and quantification (LOQ). The precision was expressed as a Relative Standard Deviation % (% RSD) and the accuracy was expressed as % Bias for each analyte. The Linearity was estimated for all amphetamines. For quantification of each analyte, the principle ions m/z 240, 254, 375, 254, 268 were used as quantifier ions for Amphetamine, MA, MDA, MDMA, and MDEA, respectively.

Results

The calibration curves were linear (r = 0.98) in the concentration range 50-2000 ng/mL for all analytes. The LODs based on signal-to-ratio (S/N) ≥ 3 were 25 ng/ml for amphetamine and its derivatives. The LOQs based on S/N ≥10 were 50 ng/ml for all analytes. The % RSD mean for all analytes which was within acceptable range (3.71-13.89). The % Bias ranged between (-4.41%) and 8.90 % for all amphetamine and its derivatives which was within the acceptable Total Error Allowable (15%).

Discussion

All analytes were found to be more stable at 4 °C and -20 °C. The % concentration changes when stored up to 132 days was ≤ 14 %. All analytes stored at room temperature were stable only for 28 days.

Conclusions

Overall performance of amphetamine and its derivatives on GC-MS instrument was acceptable for routine patients testing. Keeping in mind that the best storing condition for the samples is -20 °C.
Abstract ID 76
Phenethylamine-derived new psychoactive substances: metabolic fate and toxicological detectability of three 2C-FLY drugs.
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Aim
Innumerable new psychoactive substances (NPS) are sold as drugs of abuse. NPS are often derivatives of drugs under legislative control mimicking their psychoactive effects. This is also true for the 2C-FLY drugs, which are derived from the well-known 2C phenethylamines. The 2C-FLY drugs are also expected to induce psychostimulant and hallucinogenic effects but their methoxy groups are incorporated into rigid dihydrofuran rings. Only scarce information on their toxicity in humans is available, but 2C-FLY drugs were shown to inhibit MAO-A in vitro possibly leading to serotonin toxicity in case of overdose. Clinical and forensic toxicologists should be able to reliably detect an intake of such NPS, potentially relevant in the context of acute intoxications or unexplained deaths. On the one hand, knowledge about their metabolism and detectability in biosamples is crucial. On the other hand, knowledge about the isoenzymes involved in the metabolism is important to predict drug-drug/drug-food interactions and/or individual differences in half-life and excretion patterns. Therefore, the aim of the presented work was to investigate the metabolic fate and urinary detectability of the three NPS 2C E FLY, 2C EF FLY, and 2C T 7 FLY.

Methods
Chemical structures of the phase I and II metabolites should be elucidated in in vitro and in vivo models by means of liquid chromatography-high-resolution tandem mass spectrometry (LC HRMS/MS). In vitro incubations were performed using pooled human liver S9 fraction (pS9), while urine of male Wistar rats after high dose administration of 2 mg/kg body weight (BW) was used for in vivo measurements. Additionally, the involved human isoenzymes should be identified by performing activity screenings to detect the contribution of monoxygenases (cytochrome P450 (CYP) isoforms and FMO3), monoamine oxidases (MAO A and B), and N acetyltransferases (NAT1 and 2). Finally, the toxicological detectability in rat urine after low dose administration (0.2 mg/kg BW) should be investigated by standard urine screening approaches (SUSAs) based on gas chromatography (GC) MS, LC MSn, and LC-HRMS/MS.

Results
The main metabolic steps in vitro were hydroxylations and N acetylation. S Dealkylation of 2C T 7 FLY and deamination of the three parent compounds could only be detected in in vitro incubations. In vivo, mainly hydroxylations, N acetylation, and combinations thereof were observed. Additionally, the oxidative 2C EF FLY defluorination and its oxidation to the carboxylic acid were detected exclusively in rat urine. The 2C-T-7-FLY oxidation to sulfoxide and sulfone was detected in vitro and in vivo. A monoxygenases activity screening revealed that CYP2D6, CYP3A4, and FMO3 were involved in the phase I metabolism of the three NPS. CYP2D6 and CYP3A4 catalyzed hydroxylations and the S dealkylation of 2C T 7 FLY, and CYP3A4 and FMO3 were involved in the sulfoxide formation of 2C T 7 FLY. The monoamine oxidases and N acetyltransferases activity screenings demonstrated that MAO A, MAO B, NAT1, and NAT2 were involved. Parent compounds and/or metabolites could be detected in rat urine after low dose administration using the SUSAs by LC MSn and LC HRMS/MS. The administration of 2C EF FLY was detectable by all three SUSAs.

Discussion
The three 2C FLY drugs provided a similar metabolic pattern complemented by unique reactions in case of 2C-EF-FLY and 2C-T-7-FLY. Deamination of all derivatives could only be detected in MAO incubations after addition of the trapping agent methoxyamine but is expected to be one of the main metabolic steps in vivo. Based on the activity screenings, multiple involved enzymes could be identified but the identification of the main catalyzing isoenzyme was not possible based on the experimental conditions used. An intake of the 2C-FLY drugs is expected to be detectable by the LC-based SUSAs.

Conclusions
2C-E-FLY, 2C-EF-FLY, and 2C-T-7-FLY were metabolized by monoxygenases, MAOs, and NATs forming various metabolites. Especially the two LC-based SUSAs were suitable to detect the 2C-FLY drugs and/or their metabolites in rat urine.
Abstract ID 77
In vivo and In vitro investigations on the metabolic fate of N-ethyl-N-propyl-tryptamine, 2-aminoindane, and N-methyl-2-aminoindane.
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Aim
The aims of study were to investigate the in vivo and in vitro metabolism of the three new psychoactive substances (NPS) N-ethyl-N-propyl-tryptamine (EPT), 2-aminoindane (2-AI), and N-methyl-2-aminoindane (NM-2-AI) in a rat model as well as in pooled human liver homogenate. N-acetylation steps shall be further characterized by identifying the catalyzing enzyme isoform.

Methods
EPT, 2-AI, and NM-2-AI were administered to male Wistar rats (2 mg/kg) for toxicological diagnostic reasons and urine was collected for 24 hours. Phase I and II metabolites of the investigated substances were identified after urine precipitation by means of hyphenated mass spectrometry (MS). Precipitation was conducted by adding acetonitrile to the urine specimens. The supernatant was subsequently evaporated and finally reconstituted (2-fold concentration). To compare human and rat metabolism, the three NPS were additionally incubated with pooled human liver microsomes (pHLM) as well as pooled human liver S9 fraction (pS9). Finally, 2-AI was incubated with human N-acetyl-transferase isoform 1 and 2 (NAT1 and NAT2 respectively) to identify the isoform involved in its N-acetylation. All samples were analyzed using by liquid-chromatography (Dionex Ultimate 3000) and high resolution MS (Thermo Fisher Q-Exactive Plus).

Results
EPT was hydroxylated at the indole ring and the alkyl spacer in rat. Glucuronidation and sulfation after hydroxylation of the indole ring was also observed. After incubation with pHLM or pS9, EPT was found to be hydroxylated at one of the alkyl groups that are part of the tertiary amine. EPT was also N-dealkylated. Concerning 2-AI, the compound was in vivo hydroxylated at the indene ring and acetylated at the amino group. The incubation with pHLM and pS9 revealed an additional hydroxylation at the amino group forming a hydroxylamine. The acetylation was found to be catalyzed exclusively by NAT2. The metabolism of NM-2-AI was similar to that of 2-AI in terms of hydroxylation at the indene ring and the formation of hydroxylamines. In rat, NM-2-AI additionally underwent sulfation after hydroxylation at the indene ring. None of the amino indane derivatives or their metabolites were glucuronidated.

Discussion
Concerning the phase I metabolism of EPT, the metabolic pathways differed depending on the investigated model. In rats, EPT seemed to be primarily hydroxylated at the indole ring while incubations with human liver homogenate fractions mainly lead to hydroxylation at the amino alkyl chains as well as N-dealkyl metabolites. The parent compound was not detectable in rat urine most likely due to its extensive metabolism. Among the amino indane derivatives, only 2-AI was acetylated at the amino group. The catalyzing isoform NAT2 is known to mainly acetylate alkyl amines. Human NAT2 is also polymorphically expressed with rapid and poor metabolizers being identified, which may have an impact on the individual half-life and toxicity of 2-AI. The absence of glucuronidation steps in the metabolic pathways of amino indane derivatives is explainable by the fact that glucuronide conjugation mainly appears after aromatic hydroxylation which was not found for each of the two compounds.

Conclusions
EPT was extensively metabolized in both rat and human liver homogenate forming several phase I and II metabolites, though the individual steps are different concerning the investigated model. 2-AI and NM-2-AI were sparsely metabolized being merely hydroxylated at the indene ring. Both NPS were also transformed to one phase II metabolite each, amongst them N-acetyl 2-AI, which was exclusively formed by the polymorphically expressed NAT2. Since there are no human data available on any of the investigated compounds, the knowledge about the lack of detectability of unmetabolized EPT in rat urine and the fact that one of two metabolic pathways of 2-AI is catalyzed by a polymorphically expressed enzyme, these finding may be of special interest helping to detect the compounds in toxicological urine screenings.
Abstract ID 94

Comparison of the detection windows of heroin metabolites in human urine using online SPE and LC-MS/MS: importance of morphine-3-glucuronide.

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Aim

Heroin abuse is a serious problem that endangers human health and affects social stability. Though often being used as confirmation of heroin use, 6-monoacetylmorphine (6-MAM) has limitations due to its short detection window. Therefore, though other metabolites (morphine (MOR), morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G)) are not unique biomarkers for heroin use, it is necessary to find a metabolite with a longer detection window than 6-MAM, which can provide supporting evidence for speculating on whether to take heroin.

Methods

An automated online solid phase extraction (SPE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed to determine MOR, 6-MAM, M3G and M6G. This method was then applied to the analysis of urine samples of 20 male heroin abusers.

Results

The limits of detections (LODs) of the four metabolites were in the range of 1.25–5 ng/mL. Intra and inter-day precision for all the metabolites was 0.4–6.7% and 1.8–7.3%, respectively. Accuracy ranged from 92.9 to 101.7%. In urine samples, M3G was detected 9-11 days after admission to the drug rehabilitation institute in 40% of heroin users while MOR or M6G was not always detected. The detection window of M3G was thus the longest. Furthermore, M3G had a much higher concentration than MOR and M6G.

Discussion

We compared the detection windows of heroin metabolites in human urine and found the importance of M3G. Though the detection of M3G cannot distinguish between morphine consumption and heroin use, the long presence of M3G in human urine can provide diagnostic information with regard to heroin use in the combination with other clues. For instance, the police have found heroin seizures at the scene. M3G is positive in the suspect’s urine while 6-MAM is not detected. Heroin use can be then inferred reasonably by combining these clues if the suspect cannot provide the evidence of morphine consumption. In addition, when investigating a complicated case, the detection of M3G in a suspect’s body prompts the possibility of drug abuse. It may provide a new direction for the investigation.

Conclusions

The developed online SPE combined with LC-MS/MS method was simple, sensitive, reliable and automated. The long presence of M3G could provide supporting evidence to speculate on heroin intake combining with other clues and may also provide a new direction for the investigation in forensic cases.
Abstract ID  115
Measurement of antipsychotic medications and evaluation of drug melanin affinity in head hair samples from criminal justice population.
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Aim
Background: Recent studies have demonstrated that drugs incorporated via the blood stream would be found into inner hair compartments than those drugs incorporated via sweat, sebum and from external contamination [1]. As a consequence, the choice of extraction solvents of drugs from hair has a strong impact on hair analysis outcomes, and harmonization of extraction protocols and wash procedures is therefore more and more recommended. Moreover, evaluation of drug melanin affinity depending on the hair specimen could be of considerable importance for pharmacological reasons. Compliance monitoring of medication use is a critical factor in the management of schizophrenia patients, particularly in the criminal justice system. As relationship between medication non-adherence and violence have been already reported, it would be interesting to monitor drug use in this population by hair analysis. Aim: a) Optimization and evaluation of the extraction of 20 neuroleptics from hair; b) development of an UPLC-MS/MS method for the quantification of the following antipsychotics in hair: amisulpride, aripiprazole, chlorpromazine, clozapine, clozepine, desmethylclozapine, desmethylolanzapine, haloperidol, levomepromazine, norchlorpromazine, 7-OH-quetiapine, 9-OH-risperidone, olanzapine, pimozine, pimipamperone, quetiapine, risperidone, sertindole, sulpride, and tiapride; c) application to authentic hair samples; d) evaluation of the drug melanin affinity depending on the hair specimen.

Methods
Hair samples were decontaminated and extracted using the Psychemedics's FDA-approved protocols [2] as follows: about 10 mg of hair were decontaminated with successive washes of isopropanol and phosphate buffer/0.1% BSA. Hair samples were spiked with the internal standard and then enzymatically digested overnight with 1 mL of a solution of 0.6% dithiothreitol with 0.02 M of Bis Tris at pH 9.5 containing 0.25 unit/mL of Proteinase K. After centrifugation, the supernatant digest (fraction A) was separated from the remaining melanin hair pellet (fraction B). Fraction B was washed with water, spiked with the IS, a second digestion was carried out with di-methyl sulfoxide (DMSO) for 1h at 60°C, after pulverization. Both fractions, separately, were then submitted to a solid phase extraction with cation exchange mode cartridges. After evaporation, the samples where reconstituted in acetonitrile and injected in the UHPLC-MS/MS system. The analytes were separated on a BEH C18 analytical column with gradient elution in a total run time of 3.5 min.

The method was applied to 75 head hair samples obtained from prisoners from an antipsychotic compliance study in the criminal justice system in US carried out in 2001. All subjects gave written informed consent for a hair study based on a research protocol approved by UCLA’S Office for the Protection of Human Subjects. Hair samples, and self-reports results obtained from NIJ-funded study were identified by only a research ID number. The patients were under chlorpromazine, haloperidol, risperidone, olanzapine, or quetiapine multiple antipsychotic treatment, during incarceration. These hair samples were also analyzed for illicit drug use [3].

Results
Complete validation of the method was carried out in fraction A following international guidelines. Fraction B (second digest in DMSO) was analyzed in order to evaluate the extraction efficiency of the first digestion step and to evaluate the melanin binding affinity of remaining entrapped drugs in inner melanin layers.

Discussion
The method was applied to authentic samples and the first head hair centimeter, closest to the scalp, was analyzed. The results were evaluated in relation to the type of hair, color, porosity (following a published procedure [4]), drug melanin affinity, and prescribed dose. Information about sex and age was not currently available. Twenty seven hair samples presented high cocaine and metabolites concentrations in hair, suggesting chronic cocaine use in prison. Most of the samples were African and black color. A wide range of antipsychotic concentrations were observed, dose mg/day (d); pg/mg fraction A (A): chlorpromazine (d:50-400; A:<LOQ-1600), norchlorpromazine (A:<LOQ->1600), haloperidol (d:4-20; A:<LOQ-2902), olanzapine (d:5-20; A:<LOQ-223), desmethylolanzapine (A:<LOQ-136), quetiapine (d:4-400; A:<LOQ-2754), 7-OH-quetiapine (A:<LOQ-1448), risperidone (d:2-20; A:<LOQ-1600), 9-OH-quetiapine (A:<LOQ-296). Antipsychotics were still present in Fraction B in 33 to 100% (depending on the compound) positive fraction A samples, and at lower concentrations. Fraction B presented a general increase from B to 89% of the parent/metabolite ratio, when available. High inter-individual variability in antipsychotic relative affinity for melanin was observed, being in general, relatively higher in relaxed samples. The high affinity of melanin for some drugs could produce hair color/ethnic-bias effects with solvent based extraction methods, since, contrary to the present extraction procedure, the former do not separate the melanin from the protein fraction. The results showed that non-users of illegal drugs were just as non-compliant with prescribed medication use than the illegal drug users, indicating poor supervision with respect to the intake of their prescribed medications.

Conclusions
A fast and validated UPLC-MS/MS method is described for the quantification of 20 antipsychotics in hair. This is the first antipsychotic adherence study which uses FDA-approved decontamination and extraction methods and where the drug content in the protein and melanin fractions were analyzed separately.

Abstract ID 130
Bogumila Byrska
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Aim
Synthetic cannabinoids (SC) constitute the largest (about 30%), the most diversified and the fastest growing group of new psychoactive substances (NPS), monitored by the EMCDDA (European Monitoring Center for Drugs and Drug Addiction). They were first identified in Europe in 2008 and since then 179 new compounds belonging to this group have been reported to the EU Early Warning System. Following the rapid growth of NPS in Poland, since 2008 a number of amendments to the Act on Counteracting Drug Addiction (ACDA) have been made, aimed at reducing a phenomenon of the NPS emergence. The last but one amendment made on 1st July 2015, added to the list of controlled substances 114 new compounds. In total, counting all the previous amendments to the Act, after the 1st of July 2015, 150 NPS were controlled in Poland. The aim of this study is to show how the popularity of synthetic cannabinoids (SC) in herbal mixture products seized between 2008 - 2018 and examined in the Institute of Forensic Research in Krakow, Poland (IFR), has changed in relation to the legal changes that have been made by the subsequent amendments to the ACDA.

Methods
Herbal mixture and brown substances were homogenized and extracted with methanol. Powders were homogenized, then dissolved in methanol and in the mixture of methanol and water. For identification of synthetic cannabinoids variety of chromatographic and spectrometric methods (GC-MS, UHPLC-PDA, LC-QTOF-MS) were applied.

Results
The study of the popularity of the individual cannabinoids was carried out on the results of selected 1608 products of herbal mixtures and brown substances, resembling hashish. In the years 2008-2018, nearly 280 NPSs were identified in the IFR, of which SC constituted a group of 71 substances (22 of them were identified in seized products after 1 July 2015). As a result of the tests, it was found that:
• 75% of these products contained SC from the group of aminoalkylindoles, of which the most popular were naphthoylindoles, the presence of which was detected in 33% of all tested products;
• since 2012, SC in other structures grew in importance, and 70% of the tested products in 2015-2018 contained such cannabinoids;
• after 1 July 2015, idole-3-carboxamides (eg MDMB-CHMICA - 30%), indazole-3-carboxamides (eg AB-CHMINACA - 23%) and indole-3-carboxylates (eg NM-2201 – 22%) were the most popular.

About 20% of all the examined products contained the banned substances. An analysis of a number of detected substances, in seized products was performed. About 40% of the preparations contained two or more components. A dozen or so contained more than 4 SC.

Discussion
The products including NPS are advertised as legal substitutes of the controlled substances, causing the most similar drug effect as the banned ones. Our data show the potential danger of buying because it is unintentionally violating the law just by purchasing. The products examined very often contained mixture of synthetic cannabinoids.

Conclusions
The NSP market in Poland is characterized by high dynamics of the phenomenon. The mere addition of further substances to the list of the controlled ones turned out to be ineffective in reducing the phenomenon, because in their place the NSP producers introduce new, legal alternatives often showing a stronger effect on the CNS. The latest amendment to the ACDA (21st of August 2018) introduced many innovative solutions aimed at reducing the phenomenon associated with the NPS emergence. For the first time in the Polish drug law, besides listing individuals compounds as controlled drugs, a generic definition of four NPS groups, namely: phenethylamines, cathinones, synthetic cannabinoids and fentanyl was introduced. Reliable assessment of the effectiveness of the changes introduced will only be possible in some years later.
Abstract ID 133
Hystotoxicological Examination of A New Design Psychoactive Substance 5-MeO-MiPT.
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Aim
The initial appeal of new psychoactive substances was legally and easily available via the internet, but many of them are generally undetectable in many laboratories. In analytical toxicology these undetectable substances makes big problem in forensic cases. The psychedelic and hallucinogenic drug 5-methoxy-N-methyl-N isopropyltryptamine (5-MeO-MiPT) is an analog of the synthetic tryptamines, which are subgroup of hallucinogens, also known as Moxy. But only little is usually known about 5-MeO-MiPT, metabolism and toxicity, making the assessment of potential harms in humans. In this study, our aim is to investigate the acute toxicity of 5-MeO-MiPT in tissues and cells after injecting to mice.

Methods
Mice were divided into 4 groups: Control (n=3), 50% dimethyl sulfoxide vehicle control (n=3), injected 0.27 mg/kg 5-MeO-MiPT (n=10), injected 2.7 mg/kg 5-MeO-MiPT (n=10) groups. All injections were applied intraperitoneal (ip). After 1 hour, mice were euthanized. Blood, brains, kidneys and livers of all animals were removed and stored at -80°C until toxicological, histopathological and immunohistochemical analysis. After all samples were thawed and homogenized, blood and tissues extraction was performed with solid phase extraction method. All samples injected to liquid chromatography with tandem mass spectrometry (LC-MS/MS) system for toxicological drug analysis. For histopathological analysis, tissues were fixed 4% formaldehyde in 0.1 M PBS, then 5 μm thick sections were cut from each sample and stained with hematoxylin&eosine. For immunohistochemical analysis, after sectioning, samples were deparaffinised. Sections were tyripsinized, then circles were drawn around tissue on slides with hydrophobic barrier pen and inhibition of endogenous peroxidase activity was applied on tissues. Sections were incubated with Caspase-3 and Caspase-8 antibody in a humidity chamber overnight. Then sections were incubated with biotinylated secondary antibody and streptavidin bound horse radish peroxidase. Sections were incubated dead end colorimetric tunnel system. All samples were visualized by using Olympus BX-51 microscope.

Results
After ip injection, 5-MeO-MiPT was found 11-29 ng/g in the kidneys, 15.2-108.3 ng/g in the livers and 1.5-40.6 ng/g in the brains by LC-MS/MS. Histological examination of the mice in control, vehicle control and low dose groups showed normal architecture of hepatic lobules. Mild degree of degeneration and cytoplasm swelling of the hepatocytes and deformations of glomerulus structures of the kidneys were seen in high doses of 5-MeO-MiPT (2.7 mg/kg). When being exposed to high-dose of 5-MeO-MiPT, significant caspase-3 immunoreactivity was observed in hepatocytes which can trigger apoptosis.

Discussion
In this study, 5-MeO-MiPT distribution was shown in mice main organs (kidney, liver and brain). In another study stated that, 5-MeO-MiPT was rapidly metabolized, being almost completely removed in blood-stream after 60 min and transformed to many metabolites. The 5 most abundant metabolites were formed by demethylation and hydroxylation of the parent compound. In the same study, 5-MeO-MiPT concentrations in the blood and urine sample were found to be 160 ng/mL and 3380 ng/mL, respectively. But, in our study only parent drug 5-MeO-MiPT analyzed, metabolites concentrations measurement would be more informative. New generation of psychoactive tryptamines reported in some studies, they did not have cytotoxicity effect so would not cause cell damage. However in our study, it is seen that 5-MeO-MiPT may cause cell apoptosis in high doses, when we consider the increased immunoreactivity of caspase 3, especially in hepatocytes.

Conclusions
So far, by a majority publications for 5-MeO-MiPT have focused on receptor interaction profiles, in vitro studies and few case reports. Due to biotransformation of new psychoactive substances cannot be rapidly identified and absence of reference standards occur a general problem for laboratories that perform toxicological analysis. This study presents simple and basic histotoxicological information about 5-MeO-MiPT acute toxicity in an in-vivo mice model, which may lead to similar analysis in postmortem cases. We could not measure the metabolites 5-MeO-MiPT, this is our limitations for this study, but analyzing postmortem tissues substance levels beside blood and urine is important for forensic aspect.
Abstract ID 147
Studies on the in Vitro and in Vivo Metabolism of the New Synthetic Opioids U-51754, U-47931E, and Methoxyacetylfentanyl Using LC-High Resolution-MS/MS.
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Aim
There has been an increase in the number of so-called “New Synthetic Opioids” (NSO) in Europe and many other countries over the last decade. Data concerning toxicological or metabolic properties are only sparsely available for most of these compounds. Thus, the aim of the present work was to tentatively identify the in vitro and in vivo phase I and II metabolites of trans-3,4-dichloro-N-[2-(dimethylamino)cyclohexyl]-N-methyl-benzenacetamide (U-51754), trans-4-bromo-N-[2-(dimethylamino)cyclohexyl]-N-methyl-benzamide (U-47931E), and 2-methoxy-N-phenyl-N-[1-(2-phentylethyl)piperidin-4-yl] acetamide (methoxyacetylfentanyl) formed in pooled human S9 fraction (phS9) incubations and rat. Furthermore, both models should be compared.

Methods
phS9 fractions (2 mg/mL) were incubated for one and six hours with U-51754, U-47931E, and methoxyacetylfentanyl (25 µM, each) after addition of co-substrates necessary for common phase I and II reactions. Reactions were stopped by dilution with acetonitrile. Rat urine samples were collected over a period of 24 h after oral administration of a single dose of U-51754 (0.6 mg/kg), U-47931E (0.6 mg/kg), or methoxyacetylfentanyl (0.06 mg/kg) for toxicological diagnostic purposes. Sample preparation consisted of precipitation by acetonitrile or a solid phase extraction, both with or without prior enzymatic conjugate cleavage. All samples were analyzed by positive mode high resolution MS/MS after reversed-phase LC.

Results
The major phase I and II metabolites of the selected NSO were tentatively identified in phS9 as well as in rat urine specimens after oral administration. Concerning U-51754 and U-47931E, N-demethylation of the amine moiety, hydroxylation of the hexyl ring, and combinations of them were observed as major metabolic steps. As for methoxyacetylfentanyl, N-dealkylation to the nor metabolite, O-demethylation, and hydroxylation at the alkyl part of the molecule were found. Glucuronidation or sulfation after hydroxylation or in case of methoxyacetylfentanyl after O-demethylation could be determined additionally in the in vivo samples. In terms of differences between the in vitro and the in vivo system, sites of hydroxylation were not identical in case of the two U-substances. Less metabolites, particularly phase I metabolites formed by multiple reaction steps and phase II metabolites, could be detected after phS9. Finally, urinary biomarkers could be proposed as the N-demethyl-hydroxy and the hydroxy metabolite of U-51754, the N-demethylated metabolite for U-47931E, and the nor metabolite as well as the O-demethylated one for methoxyacetylfentanyl. Parent compounds of all substances could only be detected in minor abundance in rat urine.

Discussion
As for U-51754 and U-47931E, the results of this study were in good agreement with data of published metabolism studies of U-47700. However, phase II metabolites of this class of compounds were described for the first time even if former in vivo metabolism studies with human urine were performed. Species related differences of involved enzymes might be the reason for different sites of hydroxylation of U-51754 and U-47931E. Furthermore, metabolites found for methoxyacetylfentanyl were comparable to those detected in already published in vitro and in vivo studies as well. As already reported in previous studies, less metabolites could be found in vitro as compared to in vivo. The proposed biomarkers should be used, in addition to the parent compound, to allow the detection of an intake in human urine samples.

Conclusions
The present study demonstrated that all substances were extensively metabolized in rats. phS9 incubations formed the main phase I metabolites similar to those found in rat urine. Metabolic pathways were elucidated for all three NSO as well as possible urinary targets for toxicological urinalysis.
Abstract ID 158
Massive colchicine poisoning, when outcome is not fatal.
Anne-Sophie LEMAIRE-HURTEL
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1Chu Amiens,
2Chu Garches

Aim
Mr G., 48 years old, is treated for a microcrystalline polyarthritis with allopurinol 200mg/d and colchicine. He suffers from moderate obesity. After conflict with his wife, he consumes about 50 or 60 mg of colchicine, 8 and 16 g of paracetamol, 8g of ibuprofen and 2 g of ketoprofen with 3 glasses of whiskey. The patient is supported by the UAS and transferred to the intensive care unit. His clinical condition is stable, without loss of consciousness. In the first 24 hours, he has abundant diarrhea, vomiting and abnormal liver function progressing to sufficiency liver at day 2. From day 3, appear hematological complications with thrombocytopenia, leukopenia and anaemia. The patient is hemodynamically, neurologically and respiratory degraded to day 5 requiring intubation. Renal function gradually alters from d5 requiring daily dialysis from d9. The patient has alopecia at d15. After 22 days in unit care, he is transferred to nephrology unit for his significant renal function impairment.

Discussion
This case is really the description of a massive intoxication with colchicine. Concentrations measured are very high, in order of lethal concentrations. However, this patient has surveyed. It’s possibly due to overweight and not too Advanced age.

Conclusions
Colchicine poisoning are rare but serious with high mortality despite the prompt management of patients and the lack of antidote to date [3]. In this case with favorable outcome, only symptomatic treatments performed in intensive care have corrected the successive failures.

Methods
Colchicine assay is performed by UHPLC coupled to a triple quadrupole TSQ Quantum Access Max (Thermo Fisher Scientific) with colchicine-d3 as internal standard. The linearity of the method is between 0.5 and 50 ng / mL. Successive assays are performed the following days in order to follow the kinetics parameters, especially elimination of colchicine.

Results
The first assay (H8) shows a mass poisoning (60 ng/mL) related to a supposed ingested dose (DSI) from 50 to 60mg, or between 0.51 and 0.61mg/kg. We realized 10 assays and concentration of Colchicine in serum was still therapeutic at d17 (4.7 ng/mL).

The performed kinetic reveals a very slow purification (T1/2 = 160h) relative to the half-life time reported in the literature (14 to 30h).
Abstract ID 168
Quantitative and qualitative analysis for forensic toxicology simplified with micro volume extraction technology and high resolution accurate mass spectrometry.

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Aim
In the recent years, new synthetic drugs have appeared in the illicit market. These compounds include various analogs and newly emerged compounds with different chemical structures. One of the major concerns is the substantial inefficacy of current toxicological screening methods to identify the compounds in biological samples. The recent increase in sales and abuse of substituted drugs highlighted the need for fast and reliable screening methods of such compounds with simple sample preparation technology everybody can easily and smoothly implement. This work describes the evaluation of newly designed micro-volume QuEChERS extraction protocol with pre-packed salts in tubes using high resolution accurate mass spectrometry.

Methods
All samples were processed using a protocol described in the Micro Volume QuEChERS kit (Shimadzu Corporation, Kyoto Japan). Chromatographic separation was carried out using UHPLC system (Nexera X2, Shimadzu) with an ODS column. Mass spectrometric detection was performed in ESI positive mode with two measurement modes (full scan and MS/MS) using a quadrupole time-of-flight mass spectrometer (LCMS-9030, Shimadzu). For the data processing, LabSolutions LCMS and its formula prediction software (Formula Predictor, Shimadzu) were used. MS Workbook Suite (ACD/Labs, Toronto, Canada) were used for the confirmation of detected compounds. It can generate theoretical fragments based on the given structure and automatically assign with the given MS/MS spectra.

Results
Etizolam, Triazolam and their metabolites were selected as the test compounds which were spiked in human whole blood and serum. For the sample preparation, we used the protocol described in the instruction manual of Micro Volume QuEChERS kit and extracts were measured by full scan mode. As etizolam and triazolam are isobaric compounds, their metabolites are also isobaric compounds. Therefore, either chromatographic separation or higher resolution power is required to accurately identify and quantify these components.

Discussion
These compounds were correctly detected and identified by the extracted ion chromatograms (XIC) with the XIC window set at each theoretical mass ±2 mDa. Even though external mass calibration was applied on the whole experiment, the mass accuracy for all tested compounds was less than 1 ppm, which include all calibration levels ranging from 1 to 100 ng/mL spiked samples both in plasma and whole blood. Linear calibration curves were generated for all drugs with r² typically greater than 0.9998. These qualitative and quantitative results help to perform screening and structure confirmation experiments.

A synthetic opioid, acetylfentanyl which is well-known as a fentanyl analog was selected to evaluate the performance of formula prediction software and the mass accuracy at MS/MS measurement in biological samples. Acetylfentanyl was the first rank out of three candidates. MS Workbook Suite was used for the MS/MS spectral assignment of acetylfentanyl. Typical product ions of acetylfentanyl such as m/z 202.12190 (Mass Difference: 0.7 mDa) and 188.14317 (Mass Difference: 0.2 mDa) were correctly assigned with great mass accuracy.

Conclusions
The combination of a simple sample preparation with high-resolution accurate mass spectrometry revealed to be a useful approach to detect new compounds for forensic toxicology.
Abstract ID 170
Examination of 5 weight loss drugs in biological samples by UPLC-MS/MS.
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Aim
A women called the police and said she developed symptoms of dizziness, nausea, palpitations, after using weight loss products purchased online. Police seized a gang of people producing counterfeit drugs and seized a large number of weight-loss products. After scientifically tested, we found that the products contain metformin, phenolphthalein, sibutramine and other prohibited chemicals. Therefore, we established a rapid and sensitive determination method with LC-MS/MS for metformin, phenolphthalein and sibutramine and two metabolites N-desmethyl sibutramine and N,N-di-desmethyl sibutramine in biological samples, which can indicate the fact that the victim has taken diet food illegally added of metformin, phenolphthalein and sibutramine.

Methods
Biological samples of blood or urine were diluted with ph 6 phosphate buffer, centrifuged at high speed for 30min, and extracted by HLB solid phase extraction column for LC-MS/MS analysis. Chromatographic separation was performed in Waters BEH C18 (2.1mm×100mm, 1.7μm) column, acetonitrile and 10mmol/L ammonium acetate as mobile phase, and 5.4 min total run time with the flow rate of 0.4 mL/min and MRM mode of ms scan.

Results
We added metformin, phenolphthalein, sibutramine, N-desmethyl sibutramine and N,N-di-desmethyl sibutramine to whole blood samples for detection, and made the concentrations of them were 0.5, 1, 5, 10, 50, 100ng/mL, respectively. 5 drugs in biological samples all showed good linearity in the range of mass fraction between 0.5 and 100 ng/mL. For sibutramine, the limit of detection (LOD) was 0.01ng/mL and the limit of quantitative (LOQ) was 0.05. For the other four drugs, the LODs were 0.05ng/mL and the LOQs were 0.2ng/mL. RSDs of within-day and between-day of 5 drugs were all lower than 15% (n=6) and the extraction recoveries of 5 drugs ranges from 65% to 81%, with almost no matrix effect.

Discussion
Compared with acetonitrile precipitated protein and liquid-liquid extraction, buffer salt was used to dilute the sample and then solid phase extraction was used for pretreatment. The filler matrix of HLB extraction column contains hydrophilic and hydrophobic groups, and has a good recovery rate for both water-soluble metformin and water-insoluble phenolphthalein.

Conclusions
The method is simple, convenient and sensitive, which is suitable for the detection of metformin, phenolphthalein, sibutramine and two metabolites N-desmethylation sibutramine and N,N-di-isomethyl sibutramine in biological samples.
Abstract ID 198
The use of N-Acetyltaurine in urine as a marker for the detection of alcohol consumption in patients pre- and post-liver transplantation.

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Aim
A valid determination of alcohol consumption is important for several issues, such as driving licence, child custody, and liver transplantation. In 2012, N-Acetyltaurine (NAcT) was identified as a novel metabolite of ethanol through metabolomics-guided biochemical analysis. Only two experimental drinking studies investigating its forensic utility have been published so far. The diagnostic window of this direct alcohol marker, NAcT, has been assumed as comparable to that of ethylglucuronide in urine, although it does occur endogenously in human serum and urine samples. In this study, NAcT was compared to other alcohol consumption markers in liver transplant candidates and recipients, to evaluate whether it could improve standard alcohol screening.

Methods
In this prospective study, patients were included who presented to the outpatient transplant clinic of the UKE with either liver cirrhosis due to alcoholic liver disease, or for a yearly check-up-visit after liver transplantation. The biomarkers EtG in urine (uEtG; LC-MS/MS) and in hair (hEtG; LC-MS/MS), carbohydrate deficient transferrin (CDT; HPLC-UV), phosphatidylethanol (PEth; LC-MS/MS), and NAcT (LC-MS/MS) as well as ethanol (EtOH; GC-FID), methanol (MeOH; GC-FID) were tested and compared with patients’ questionnaire reports. NAcT concentrations above the endogenous level of 2300 µg/L (maximum), normalized to a creatinine concentration of 1 mg/L, were regarded as positive.

Results
Of 51 pre- and 61 post-transplant patients, 35/112 (31%) tested positive for at least one alcohol marker. NAcT was positive in 37% (13/35), PEth in 57% (20/35), EtOH in 11% (4/35), MeOH in 14% (5/35), CDT in 14% (4/29), uEtG in 34% (11/32), hEtG in 64% (16/25) patients, respectively. Not all patients’ urine and hair samples were available.

In four patients, in addition to NAcT, at least uEtG and PEth were also positive, revealing recent alcohol consumption. In one patient, NAcT and MeOH were both positive. In two patients, NAcT and hEtG were the only positive markers. NAcT alone was positive in six patients (including the self-report); in five of the 35 patients, no NAcT results were available.

Discussion
A positive result for NAcT was obtained in 11% of all patients (13/112). In seven of these NAcT-positive cases, at least one other marker was positive. Therefore, one could regard these results as ‘true positive’. In six cases, no other marker was positive and the patients denied alcohol consumption. Therefore, the results were regarded as ‘false positive’. There was no correlation between NAcT and EtG in urine, as in nine of the 13 NAcT-positive cases, uEtG was negative.

Conclusions
Due to the high ‘false positive’ rate and implausible results (NAcT in combination to hEtG or MeOH as the only positive parameter), NAcT cannot be recommended as a reliable marker for the detection of alcohol consumption amongst transplant patients. On the basis of our data, it cannot be judged if NAcT will be more valid for patients without underlying liver disease.
Abstract ID  200
Norcarfentanil may also come from remifentanil treatment.
Helene Eysseric
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Aim
Norcarfentanil is the major metabolite of carfentanil, the most powerful fentanyl analog among the new synthetic opioids. We present a case where norcarfentanil detection was wrongly attributed to carfentanil misuse and then explained by remifentanil treatment.

Methods
A 57-year old man was at a music festival when he went to the first aid team, complaining of dizziness and faintness. He had a heart attack a few minutes later while being examined. Cardiopulmonary resuscitation (CPR) was given for 45 min and necessitated four external electric shocks and use of both epinephrine and amiodarone to stabilize the patient. At that point, the patient presented bilateral mydriasis. He was then admitted to a cardiovascular intensive care unit (CVICU) and received morphine. He died after 6 days in CVICU without clinical improvement.

For toxicological investigations we disposed of two plasma samples on day 1 and 2 of hospitalization and a urine sample on day 2. In the first step, screenings by immunoassay and by GC-MS in full scan mode were performed in urine. Confirmation step included a targeted analysis on 15 synthetic opioids by LC-MS/MS in plasma and urine. 200 µL of sample were added to 200 µL of precipitant reagent (sulfosalicylic acid 500 mg/mL) with deuterated internal standard (fentanyl-D5). After centrifugation, supernatant was injected in a 2D-chromatographic system (oasisHLB/Xselect Waters) coupled with API4000 ScieRx. MRM transitions were m/z 395.2/335.3 and 113.1 for carfentanil; m/z 291.2/142.0 and 231.0 for norcarfentanil; m/z 377.1/113.2 and 317.2 for remifentanil and 342.3/188.2 for fentanyl-D5. Opiates and metabolites were also quantified by LC/MS-MS.

Results
Qualitative urine immunoassays were negative for amphetamine, MDMA, THCCOOH, cocaine, methadone and buprenorphine but strongly positive for opiates, justifying a targeted analysis. LC-MS/MS opiate determination indicated the presence of morphine (1,660 µg/L) and codeine (< 5 µg/L) in the urine sample from day 2, whereas no opiates were detected in the plasma sample from the same day. GC-MS urine screening revealed the presence of propofol, laudanosine, lidocaine, morphine, codeine and norcarfentanil. With the synthetic opioids targeted LC-MS/MS technique, carfentanil has not been detected in any sample; norcarfentanil concentrations were respectively at 0.58; 0.08 and 10.5 µg/L in plasma day 1; plasma day 2 and urine day 2; and remifentanil concentrations at 3.12; 0.17 and 70 µg/L.

Discussion
The presence of norcarfentanil revealed by GC-MS screening was firstly attributed to carfentanil misuse. The targeted analysis showed an additional presence of remifentanil but no trace of carfentanil. We had carried out further questioning of medical team, which revealed that the patient received remifentanil on the first day. The presence of norcarfentanil in the biological samples was finally explained by remifentanil administration as part of medical treatment.

Conclusions
The presence of norcarfentanil in the biological matrices may be due to metabolism of the remifentanil administered as part of the medical treatment, justifying the exclusion of carfentanil misuse. Indeed, norcarfentanil is a common metabolite of remifentanil which is only used for anesthesia in medical care units, but also of carfentanil, which is an ultra potent illicit fentanyl analog.
Abstract ID 211
Evaluation of cannabinoids analysis in the urine and hair of users taking long-term use of hemp products.
Dongeon Park
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Aim
Since the hemp seed has been licensed as a food ingredient since 2015 in Korea, the circulation of hemp seed products has rapidly increased. The presence of trace residual delta-9-tetrahydrocannabinol (THC), the main psychoactive ingredient in marijuana, in food products incorporating hemp seed and seed derivatives has raised concerns on the possibility of abuse. In order to distinguish between the consumption of hemp seed products and abuse of hemp for hallucination purposes, the need for data analysis of the cannabis components from biological samples of people taking hemp seed products emerged.

Methods
In order to choose the products to be taken to the subjects, we analyzed only the THC content in frequently distributed hemp seed products using liquid extraction with methanol for preparation and gas chromatography/mass spectrometry (GC/MS) analysis through previous study. Among the products with relatively high THC content, we selected one domestic hemp seed, one foreign cannabis seed, and foreign hemp seed oil capsule. Thirty-two THC-naive adults took these products containing 11.14 μg to 124.8 μg of THC daily for 12 weeks divided into three groups. Urine specimens were collected prior to the first ingestion and at 7-day intervals until two weeks after the last dose. Urine specimens were screened for cannabinoids through the Kinetic Interaction of Microparticles in a Solution (KIMS, Cobas C311), confirmed for cannabinol (CBN), cannabidiol (CBD) and 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (cTHC) via gas chromatography-mass spectrometry (GC-MS). Hair specimens were collected before the first ingestion and at 7-day intervals until two weeks after the last dose. Urine specimens were screened for cannabinoids through the Kinetic Interaction of Microparticles in a Solution (KIMS, Cobas C311), confirmed for cannabinol (CBN), cannabidiol (CBD) and 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (cTHC) via gas chromatography-mass spectrometry (GC-MS). Hair specimens were collected before the first ingestion, 6 weeks after starting and 2 weeks after the last dose. The THC, CBN, CBD and cTHC in hair specimens were determined through the liquid chromatography-tandem mass spectrometry (LC-MS/MS). The GC-MS and LC-MS/MS methods for THC, CBN, CBD and cTHC in biological specimens were developed and validated.

Results
Twenty-six urine specimens that screened positive for cannabinoids at the 10 ng/mL cutoff by Cobas C311, were negative for CBN, CBD and cTHC in confirmation by the GC-MS at the 1 ng/mL. None of the hair specimens was confirmed positive for THC, CBN, CBD and cTHC through LC-MS/MS, except for the specimen that was collected from the subject that ingested product containing 11.14 ug/day of THC for which the CBN peak was detected (below the quantitation limit 20pg/mg CBN).

Discussion
In this study, three types of hemp products which are relatively high in THC content among the domestic and foreign products in Korea were used. Since the purpose of this study is to investigate the influence of the hemp products on urine test when taken at the usual dose as food products, the exact contents of individual components such as THC and THC acid A were not considered. Because hemp seed products distributed in Korea are strictly controlled by the acceptance criteria as domestic food raw materials and we used products containing relatively high THC among these in the same experimental conditions, it is considered that the results of this study can be applied to the urine and hair of people who took the commercial cannabis products in Korea. CBN was detected in the hair of one volunteer, but its concentration was much lower than the limit of quantitation. So, it is considered that it cannot give an important meaning. Moreover, the possibility of detecting THC, CBN, CBD and cTHC from the hair of a person who has taken a commercial hemp product is low.

Conclusions
Based on our study, the daily ingestion of hemp products that are distributed in Korea does not produce positive urine cannabinoids results through the GC-MS or LC-MS/MS. It is thought that there is a significant meaning in preparing a justifiable basis for a case in which abusers claim that it is the result of taking hemp products to deny their positive results.
Abstract ID 214
Eugenol ingestion: A case report.
Mila Lovrić
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Aim
The aim of this study was to discover the cause of clinical picture in a 13-month-old girl who was admitted to the pediatric emergency unit due to loss of consciousness in a day-care center. Two days before this episode, she had symptoms of acute respiratory infection. She did not ingest substances of unknown origin.

Methods
Targeted toxicological screening (immunochemistry) for amphetamine, opiates, methadone and benzodiazepines was applied. For general toxicological screening, after liquid-liquid extraction in pH=9, gas chromatography-mass spectrometry (column: Crossbond 5% diphenyl/95%dimethyl polysiloxane, 30 m, 0.25 mm i.d. and 0.25-µm film thickness, using helium at a flow rate of 1.56 mL./min as the carrier gas.) was used. Eugenol was detected by database comparison and confirmed by GC-MS analysis of clove essential oil for pharmaceutical use.

Results
Laboratory findings indicated increased urea, AST and ALT levels in serum. Targeted toxicological screening was negative for amphetamine, opiates and methadone and positive for benzodiazepines (serum, urine; the patient received mirtazapine in ER.). General toxicological screening (GC-MS- urine) revealed eugenol and mirtazapine in the urine sample. EEG has indicated the possibility of taking a medication.

Discussion
Eugenol is a natural phenolic compound with flavoring properties and is the most abundant compound of clove essential oil. It is used in perfumes, flavorings, and essential oils. It is also used as a local antiseptic and anaesthetic (dental profession). Eugenol toxicology is connected with contact dermatitis, metabolic acidosis, coma, seizures, hypoglycemia and liver failure. After administration of N-acetylcysteine, the patient’s AST and urea levels dropped to normal within two days. ALT levels normalised within five days.

Conclusions
Eugenol found in urine may be a reason for consciousness disorder, elevated urea, AST and ALT levels in serum, and for EEG changes. However, why eugenol occurred in the urine remains unknown. Our GC-MS analysis is qualitative only so we cannot be sure if eugenol caused such clinical picture. After five days the little girl was released from hospital. One month later the girl was in hospital for follow-up examination where consciousness disorder accompanied by convulsive elements were detected and she was diagnosed with epilepsy.
Abstract ID  215
Ingestion of magic mushrooms: A case report.
Mila Lovrić

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Aim
The aim of this study was to detect and confirm the cause of symptoms in two boys aged 15 and 16 years who were admitted to the emergency department.

Methods
Targeted toxicological screening (immunochemistry) for amphetamine, cannabinoids, opiates and benzodiazepines was used. For general toxicological screening, gas chromatography-mass spectrometry (GC-MS) was used.

Results
The 15-year-old boy was hyperactive and paranoid, had fear of death, he was calling his friend „daddy” and was completely in his own world. At the same time the 16-year-old boy was lying down on the kitchen floor pretending that he was talking with someone on the phone. Later he became aggressive. In ER, the 15-year-old boy was hyperactive, aggressive, out of contact; the 16-year-old boy was lying down, occasionally answered questions coherently, and had tachycardia. A cup with a liquid smelling of mushrooms and cigarettes and weed was found in the room. Targeted toxicological screening was negative for amphetamine, opiates and benzodiazepines and revealed positive cannabinoids in the 15-year-old boy. General toxicological screening (GC-MS) revealed psilocin in the urine samples of both boys. General toxicological screening is qualitative, but psilocin peak area on the chromatogram was about ten times higher in the younger patient.

Discussion
Clinical picture of patients was different (one was aggressive and another wasn’t), which confirms that the mind-altering effects of psilocin are highly variable and subjective. The 15-year-old patient was also positive for cannabinoids so his clinical picture was not the result of psilocin ingestion only. He also had about 10-fold greater amount of psilocin in the urine.

Psilocin was a hallucinogenic alkaloid isolated from Psilocybe mushrooms. It is found in most psychedelic mushrooms together with its phosphorylated counterpart psilocybin. Psilocybin is often the major component of magic mushrooms and is after ingestion rapidly converted by dephosphorylation into psilocin. The majority of psilocybin users experience a pleasant alteration in mood, but some experience panic and dysphoria. These effects typically last from three to eight hours. The psilocin found in urine was the cause of clinical symptoms in the described patients. The following morning the patients were without any symptoms of magic mushroom ingestion.

Conclusions
Psilocin was confirmed in the urine sample, and qualitative confirmation along with the clinical condition of the patient is sufficient to confirm the poisoning and the need for care of patients in an emergency department. This is a valuable finding for us in clinical toxicology because this is the first case of mushroom poisoning since the GC-MS method for general toxicological screening has been applied for 24 hours. In types of poisoning that occur rarely it is, without practical experience, rather difficult to send a toxicological finding to a doctor who expects it to treat a patient, especially during night duty.
Abstract ID 231
Optimisation of a fast and easy quantification method for 54 benzodiazepines & Z-drugs, including 20 designer benzodiazepines, in plasma.

Maarten Degreet
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Aim
In the last decades, hypnotics including the benzodiazepines/Z-drugs have earned their place in our society for therapeutic applications such as battling anxiety and sleeping disorders. However their use comes with important side effects, withdrawal symptoms, dependency and unsafe traffic situations. On the other hand their illegal use is rapidly rising as well, predominantly in combination with other drugs such as alcohol and opioids where the importance of their added suppressant effect is often overlooked. We aimed to set up a cheap, easy and time-efficient (for both sample preparation and instrumentation used) method to accurately detect and quantify most of the commonly prescribed benzodiazepines, including 20 new designer ones, in plasma.

Methods
In a first step the sample preparation was optimised based on 3 different sample volumes: 100 µL, 200 µL and 500 µL plasma. In-house techniques included protein precipitation using ACN, liquid-liquid extraction (LLE) using MTBE or hexane/EtAc (70:30), and mixed-mode (C8/SCX) solid-phase extraction (SPE) using EtAc + NH4OH (5% v/v) or DCM/isopropanol (80:20) + NH4OH (2% v/v) as elution solvents. Mini-QuEChERS were tested with 4 different extraction solvents (ACN, DCM, MTBE and hexane) each with and without a secondary clean-up step using primary and secondary amino sorbents and MgSO4.

Samples were injected on a liquid chromatograph (LC) coupled to a triple quadrupole mass spectrometer (QQQ). A Zorbax Eclipse Plus C8 column (2.1 x 150 mm, 3.5 µm) provided the necessary separation in combination with a mobile phase gradient going from 95% mobile phase A (water + 0.1% formic acid v/v) to 95% mobile phase B (10:90 water:ACN + 0.1% formic acid v/v) in 9.4 minutes. The total run time was 11.4 minutes. Three transitions (2 for deuterated internal standards) were monitored per compound in the dynamic multiple reaction monitoring mode (ESI+). For compounds where deuterated analogues were not available, both structural and retention time matched internal standards were investigated.

Results
During extraction optimisation, the LLE and the SPE methods showed similar relative recoveries, varying between 70% and 90% depending on the compound under observation. Standard deviations based on 4 repeats were generally less than 10%. The QuEChERS approach showed less reproducible and less efficient recoveries.

The method was further validated according to the European Medicines Agency guidelines. Linear calibration curves were obtained for most compounds that had a deuterated analogue as internal standard, quadratic curves for the other compounds. Accuracy and precision was within the set ranges for all compounds, except for pivoxazepam where the between-batch variability was around 20%. On average no significant difference in accuracy and precision was found between the use of structurally matched internal standards (102% ± 5%) and retention time based internal standards (101% ± 6%). In-depth evaluation of the extraction method used showed recoveries of 78% ± 8% on average, with the exception of pyrazolam where a recovery of only 41% ± 10% was seen.

Discussion
A sample volume of 100 µL proved insufficient for the sensitivity needed. Due to the low concentrations of the intended calibration curves, it was chosen to continue with 500 µL sample volume. The QuEChERS techniques initially showed promising results for even the lowest sample volume. However, both the extraction efficiency and reproducibility were less than for the other techniques. Implementation of an additional clean-up step did not significantly improve the outcome results. As both LLE and SPE methods had equal extraction efficiencies, the LLE method was chosen based on time-efficiency and similarity with other methods.

The LC-QQQ method proved accurate and robust for all but a few compounds where the requirements for the LLOQ could not be met. Mainly due to its low recovery, the instrument sensitivity was insufficient for pyrazolam, which was excluded from the final method. Internal standards were matched on either retention time or structural similarities. The effect on the validation parameters was only minor. Previously described problems with long-term stability of the benzodiazepines were noted as well during 3-month stability studies at -20 °C.

Conclusions
The presented method successfully quantifies most commonly encountered benzodiazepines, including many designer ones, in plasma. Because of its simple sample clean-up, relatively short chromatographic run time and simple data analysis, this method can readily be implemented in both therapeutic drug monitoring and forensic research.
Abstract ID 239
Amphetamine and methamphetamine overdoses analytically confirmed: clinical characteristics of the patients attending during 2013-2018 in the Balearic Islands.

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Aim
To investigate if exist differences between clinical and toxicological features in intoxications involving amphetamine (AMP) and methamphetamine (MAMP) among patients presenting to Emergency Departments (ED).

Methods
Design: Retrospective observational study of AMP and MAMP overdoses presented to ED between 2013 and 2018. Inclusion criteria: Patients with declared consumption or clinical findings consistent with sympathomimetic toxicity and AMP or MAMP confirmed in urine samples. Exclusion criteria: Patients who were not acute overdozed or prescribed AMP. Clinical characteristics of the first episode were included. Methods: Urine drug screen was performed by immunoassays (DRI®Abbott Assay or Alere Triage®TOX Drug Screen; cut-off AMP/MAMP=1,000ng/mL). Serum ethanol was measured by an alcohol dehydrogenase method. Positive presumptive results were confirmed by gas chromatography-mass spectrometry. Data analyses: Patients were divided in AMP and MAMP groups. Demographic and clinical features at admission, reasons for ED presentation, toxicology results, and patient outcome were compared. Data are presented as mean±standard deviation or number (percentage,%). Comparisons were made using the chi-square test and an unpaired Student t-test respectively. The statistical significance differences was established as p<0.05.

Results
Clinical features: 121 patients were included. 86 (71.1%) in the AMP and 35 (28.9%) in the MAMP group. The ED presentations increased, from no cases to 53 cases. The comparisons between AMP and MAMP groups were the following. Demographic variables: age: 32.3±8.2 and 28.6±8.0; sex (men): 62 (72.1%) and 32 (91.4%); Spanish nationality: 53 (61.6%) and 10 (28.6%); multiple ED presentations: 6 (7.0%) and 3 (8.6%); co-exposure to ethanol and/or other illicit drugs: 71 (82.6%) and 21 (60.0%). Differences were found between sex, Spanish nationality and co-exposure to ethanol and/or other illicit drugs. In mono-intoxications cases, differences were found in non-Spanish nationality (5 (33.3%) and 13 (92.2%)). In MAMP group, 5 (35.7%) cases were Filipinos. Reason for ED presentation: agitation: 13 (15.1%) and 6 (17.1%); conduct disorder: 11 (12.8%) and 0; decreased level of consciousness: 10 (11.6%) and 2 (5.7%); toxic-induced psychotic episode: 7 (8.1%) and 7 (20.0%); seizures: 8 (9.3%) and 0; traumaism: 8 (9.3%) and 1 (2.9%); aggression: 5 (5.8%) and 2 (5.7%); palpitations: 1 (1.1%) and 7 (20.0%); autolytic attempt: 4 (4.7%) and 2 (5.7%); anxiety: 3 (3.5%) and 2 (5.7%); chest pain: 4 (4.7%) and 0; others causes: 8 (9.3%) and 5 (14.3%); and unknown: 4 (4.7%) and 1 (2.9%). Differences were found between conduct disorder and palpitations. Clinical features at admission: tachycardia (≥100bpm): 30 (34.9%) and 14 (40.0%); agitation: 23 (26.7%) and 3 (8.6%); mydriasis: 19 (22.1%) and 9 (25.7%); decreased level of consciousness (GCS<15): 18 (20.9%) and 4 (11.4%); seizures: 5 (5.8%) and 0; hypertension (SBP≥140mmHg): 13 (15.1%) and 11 (31.4%); rhabdomyolysis (CK>250U/L): 14 (16.3%) and 7 (20.0%); elevated troponins (>10pg/mL): 8 (9.3%) and 0; hypokalemia (≤3.3mmol/L): 2 (2.3%) and 4 (11.4%); hyperthermia (>39ºC): 8 (9,3%) and 0; hallucinations: 6 (7.0%) and 6 (17.1%); delusions: 7 (8.1%) and 0; disorganized speech or behavior 2 (2.3%) and 4 (11.4%); diaphoresis: 4 (4.7%) and 0; stroke: 1 (1.2%) and 1 (2.9%); cardiac arrest: 0 and 1 (2.9%); and altered EKG report: 2 (2.4%) and 1 (2.9%). Differences were found between agitation and hypertension. In mono-intoxication cases, the most frequent clinical features were: 1) in AMP group: agitation (33.3%), hallucinations (26.7%) and tachycardia (20.0%); 2) in MAMP group: tachycardia (42.9%), mydriasis (28.6%) and hypertension (28.6%). No differences were found. Episode outcome: discharge from General ED: 53 (61.6%) and 23 (65.7%); discharge from Psychiatry ED: 5 (5.8%) and 6 (17.1%); admission to ICU: 7 (8.1%) and 2 (5.7%); admission to Psychiatry Unit: 10 (11.6%) and 1 (2.9%) admission to Neurology Unit: 2 (2.3%) and 1 (2.9%); admission to other services 1 (1.2%) and 1 (2.9%); death: 0 and 1 (2.9%). No differences were found.

Toxicology results: 986 urine samples resulted presumptively positive for amphetamines and only in 191 (19.4%) were positively confirmed. 70 samples (36.6%) were excluded. Mono-intoxication was less frequent in AMP group than in MAMP group (15 (17.4%) and 14 (40.0%);p=0.017). Cannabis was the most prevalent drug in poly-intoxication cases and was more frequent in AMP group than in MAMP group (39 (45.3%) and 6 (17.1%);p=0.004). After cannabis, the most detected drugs were cocaine>ethanol>MDMA. Morphine, ketamine, methadone and GHB were also detected. False-positive rate was 80.6%. The compounds likely to cause false-positive results identified in 69.3% of samples. Since this was a retrospective study, only urine samples were available for confirmation and serum levels of AMP/MAMP was not available.

Discussion
AMP related overdoses were associated to Spanish nationality, poly-intoxications, consume of cannabis, agitation and conduct disorder, while MAMP poisonings were related to men, non-Spanish nationality, mono-intoxications, palpatations and hypertension. Most patients were discharged from ED.

Conclusions
Differences between demographic and clinical features in AMP and MAMP intoxications exist. Clinicians must be aware the deleterious effect that these drugs produce, especially on mental and physical patient health. Increased availability and use impact on ED.
Abstract ID 253
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Aim
Adderall is a sympathomimetic amine with CNS stimulant activity indicated for the treatment of attention deficit hyperactivity disorder (ADHD) and narcolepsy. Tablets contain d-amphetamine and l-amphetamine salts in the ratio of 3:1. Amphetamines block the reuptake of norepinephrine and dopamine into the presynaptic neuron, and increase their release into the extra-neuronal space, although the mechanism of therapeutic activity in ADHD is not known. Our clinical experience has shown that many patients compliant with Adderall therapy had very low but detectable concentrations of methamphetamine relative to the concentration of amphetamine. It has been well documented that pharmaceutical preparations of opioids have allowable levels of other opioids, albeit at percentages typically below 1%. Our hypothesis was that a similar phenomenon exists for Adderall and in which a small amount of methamphetamine is present as an impurity or by-product from the amphetamine manufacturing process which could explain the very low concentrations of methamphetamine observed in compliant patients taking Adderall.

The aim of this study was to determine if Adderall contained trace amounts of methamphetamine, or other amphetamine type stimulants.

Methods
Adderall tablets were dissolved in methanol (and 10% water) to make a stock solution of 1 mg/mL. This solution was used to make further dilutions to obtain various concentrations used for the analysis; 1) to verify the amount of amphetamine in the pill material, and 2) to be able to quantitate and verify the presence other amphetamine type stimulants that may be a pharmaceutical impurity.

In order to utilise our existing laboratory procedure, standards, QC and diluted pills were spiked into drug free urine and extracted using SPE (Clean Screen columns). The drugs were eluted using dichloromethane:isopropyl alcohol:ammonium hydroxide (78:20:2) and dried under nitrogen. The analytes were derivatised with HFAA and N-butyl chloride. Samples were reconstituted in chloroform and analysed on an Agilent 6890 GC coupled to a 5973 MS in SIM mode.

A standard curve was prepared from 5.0 – 1,000 ng/mL for amphetamine, methamphetamine, methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), and pseudoephedrine, and their corresponding isotopically labelled internal standards. Controls were prepared in house from a different stock standard at 10.0, 62.5 and 750 ng/mL.

Results
The Adderall tablets (8) contained small amounts of methamphetamine and when expressed as a percentage of the amount of amphetamine in the tablet, the results ranged from 0.0010% to 0.0088%. No other amphetamine type stimulants were identified. In comparison, the commercially available amphetamine material used to prepare the standards contained no identifiable methamphetamine.

Discussion

Conclusions
Similar to what has been described with opioids; pharmaceutical preparations of Adderall contain very small amounts of methamphetamine. This could be an explanation for the observations of trace amounts of methamphetamine being detected in patients who are complaint with Adderall therapy and may not necessarily indicate polydrug use.
Abstract ID 257
Microextraction by packed sorbent to determine methadone and EDDP in hair samples: a new approach for sample clean-up.
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Aim
This work describes the development and validation of a novel analytical method for the simultaneous determination of methadone and the main metabolite (EDDP) in hair samples by gas chromatography-tandem mass spectrometry (GC-MS/MS) using microextraction by packed sorbent (MEPS), a miniaturized SPE approach, for sample clean-up.

Methods
Previously washed hair samples were cut into small fragments and 50 mg was weighed into glass tubes. One mL of 1M sodium hydroxide was added, and incubation took place at 50 °C for 45 minutes. The extracts were centrifuged at 3500 rpm (15 minutes), were mixed with 100 μL of 20 % formic acid, and 20 μL of internal standards working solution was added. The steps for sample clean-up were previously optimized, and the final conditions were as follows: conditioning (3 cycles of 250 μL of methanol and 3 cycles of 250 μL formic acid 2%); sample load (9 cycles of 150 μL); wash (150 μL of 3.36% formic acid); and elution (6 cycles of 100 μL of ammonium hydroxide 2.36% (in methanol). The extracts were evaporated to dryness under a gentle nitrogen stream, and were afterwards dissolved in 50 μL of methanol, from which 2 μL was injected in the chromatographic system (GC-MS/MS). The separation of the analytes was achieved using a capillary column of fused silica (30 m × 0.25 mm, I.D., 0.25 μm) with 5% phenylmethylsiloxane (HP-5 MS) supplied by J & W Scientific (Folsom, CA, USA). The oven temperature started at 150 °C for 2 min, followed by an increase of 20 °C per minute until 300 °C and was maintained for 3 min. The total time of the chromatographic run was 12.50 min. The injection volume of the sample was 2 μL in splitless mode, and the injector and transfer line temperatures were set at 220 and 280 °C, respectively. The carrier gas, helium, was set at a constant flow rate of 0.8 mL/min. In the mass spectrometer, the helium flow was 1.5 mL/min and that of nitrogen was 2.5 mL/min in the collision cell in electron impact mode, with a current of 35 μA and electron energy 70 eV. Data were acquired on multiple reaction monitoring (MRM) mode.

The method was validated following the standards of the Food and Drug Administration and the Scientific Working Group of Forensic Toxicology. The studied parameters included selectivity, calibration model and linearity, limit of detection and limit of quantification, precision, accuracy and recovery.

Results
Linearity was obtained from the lower limit of quantitation (LLOQ) up to 5 ng/mg, with all target compounds revealing determination coefficients greater than 0.99. The LLOQs were 0.025 ng/mg, and recoveries greater than 70% were obtained for both compounds. Precision and accuracy were acceptable, namely coefficients of variation typically below 15% and relative errors within a ±15% interval from the nominal concentration were obtained. Drug free hair samples were extracted after the extraction of the upper limit of quantitation (ULOQ), and no signs of carryover were detected.

Discussion
The obtained limits can be considered quite satisfactory, especially when compared to the available literature on the subject. Many authors adopt hair incubation as a unique sample preparation procedure, not considering the additional clean-up of the resultant extract. This results in lower losses of the analytes, but also in dirtier extracts, hence compromising chromatographic analysis. For this reason, SPE is a traditional and widely implemented clean-up procedure for hair specimens, however the great volumes of organic solvents usually required with this technique might be considered a disadvantage. A miniaturized procedure is described to pre-concentrate methadone from hair samples by means of solid-phase microextraction (SPME), however presenting higher LOQ. The influence of different hair types/colours/treatments (bleaching etc) was not evaluated.

Conclusions
MEPS is a good alternative to classical clean-up procedures, with low consumption of organic solvents, also allowing the re-utilization of the sorbent (over 100 extractions are possible using the same cartridge) and thus reducing the cost per analysis. It is noteworthy that this is the first time that the combination of MEPS and GC-MS/MS is used for the determination of these compounds in hair, making it an advantageous tool for their screening in clinical and forensic scenarios.
Abstract ID 258
Analytical approach to determine antipsychotics drugs in oral fluid by dried saliva spots.

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Aim
The aim this work was the use of dried saliva spots (DSS) as an alternative to the conventional sample preparation techniques. The present work describes the optimization and full validation of a sensitive method to determine several antipsychotic drugs (chlorpromazine, levomepromazine, cyamemazine, clozapine, haloperidol and quetiapine) in oral fluid using the DSS sampling approach and gas chromatography-tandem mass spectrometry (GC-MS/MS).

Methods
Sample extraction was carried out by spotting the oral fluid sample (50 µL) onto the filter paper card (Whatman™ 903 protein saver cards). Chromatographic analysis was performed using an HP 7890A GC system equipped with a triple quadrupole mass spectrometer (model 7000B), both from Agilent Technologies. Chromatographic separation was achieved using a 5% phenylmethylsiloxane column. In the present work, the type of solvent and volume, drying time of the DSS and homogenization time were evaluated and optimized. The final procedure was as follows: 50 µL of oral fluid was applied in the card and dried for 1 hour at 36 ºC. Subsequently, 2 mL of methanol (pH = 5.0) with 25 µL of IS solution was added and the extraction was performed with a rollermixer at 70 rpm for 5 minutes. The samples were then centrifuged for 15 min at 3500 rpm. The extract was evaporated to dryness under a gentle nitrogen stream and was afterwards derivatised with 50 µL of MSTFA with 5% TMCS for 2 minutes in a microwave oven at 800W. A 2 µL aliquot of the derivatized extract was injected into the GC-MS/MS system.

Results
The method was fully validated, and the included parameters were selectivity, linearity, limits of quantification, precision and accuracy, stability, dilution integrity and recovery. The method was linear for all compounds from 10-400 ng/mL, except for haloperidol (5-100 ng/mL), presenting coefficients of determination higher than 0.99. Inter- and intra-day precision and accuracy were in conformity with the criteria usually seen in bioanalytical method validation, i.e., coefficients of variation were lower than 15% and an accuracy of 15% or better for all studied drugs. The recoveries obtained with this miniaturized technique ranged from 63 to 97%. The described method was applied in routine analysis of the target APDs in authentic oral fluid samples belonging to patients under treatment in the psychiatric center of the Hospital Cova da Beira, Covilhã, Portugal.

Discussion
The obtained limits can be considered quite satisfactory, especially when compared to the available literature on the subject. Also, the proposed extraction procedure allowed great recoveries with similar or greater values than those obtained with classic techniques such as solid phase extraction. Concerning the stability, the analytes were found stable on the dried saliva spot at room temperature for 4 days, and this period could even be extended to 8 days for quetiapine and haloperidol.

Conclusions
The method was considered a good alternative to the conventional techniques to be applied in clinical and toxicological analyses, specially taking into account the extremely low sample volume used (50 µL). This is the first developed method for the determination of antipsychotic drugs in oral fluid using this sample approach. The use of this specimen to determine antipsychotic drugs is important in therapeutic drug monitoring, and evaluation of these drugs compliance.
**Abstract ID 265**

**Development of dilute-and-shoot to detect 124 abused drug and metabolites in urine using ultra-high-performance liquid chromatography–tandem mass spectrometry.**

**Da-Peng Yang**

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**Aim**

New psychoactive substances (NPS), especially synthetic cathinones, have been found increasingly in the last decade, leading the negative results from urine drug test and finally, easily evading criminal sanctions. With a view to identify emerging NPS, a rapid and sensitive dilute-and-shoot method coupled to ultra-high-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) is developed for screening and quantification simultaneously of 124 drugs and their metabolites, including types of amphetamine, opium, cannabis, cocaine, ketamine, benzodiazepines, barbiturates and emerging drugs.

**Methods**

An aliquot of 100 µL pooled urine is spiked with 50 µL of internal standard solution (50 ng mL-1 of amphetamine-d8, GHB-d6, and 7-aminoclonazepam-d4), diluted with 50 µL of standard solution at 11 different levels (0.005, 0.02, 0.05, 0.2, 0.5, 2, 5, 20, 50, 100, and 200 ng mL-1). In analysis, methanol:water (2:98 v/v) containing 0.1% formic acid and methanol containing 0.1% formic acid are used as mobile phase A and B, respectively. A gradient elution program is constructed as follow: 0-10 min, 5-90% B, a flow rate from 0.5 to 1.0 mL min-1; 10-12 min, 90-95% B, a constant flow rate at 1.0 mL min-1; 12-12.5 min, 95% B, a flow rate from 1.0 to 0.5 mL min-1; 12.5-13 min, 95-5% B, a constant flow rate at 0.5 mL min-1; 13-15 min, re-equilibrium at initial condition. Finally, the analytical process is validated in regard to accuracy, precision, linearity of calibration curve, limit of detections (LODs), and matrix effect after UPLC-MS/MS analysis.

**Results**

The linear calibration curves range from 0.005 to 200 ng mL-1 with acceptable coefficients of determination (R2 > 0.991). LODs are from 0.005 to 0.5 ng mL-1. The accuracy of 20 ng mL-1 are between 80.1 and 114.9%. The relative standard deviations range from 0.1 to 13.5% for intraday precision (n = 5) and 0.2 to 14.7% for interday precision (n = 15). All analytes manifest matrix effects of 73.2 to 108.6%.

**Discussion**

The dilute-and-shoot method has been applied to analyzing 660 urine samples. With simple sample preparation, only 1 min is needed for each sample. One hundred of 124 analytes give LODs < 0.02 ng mL-1, meaning that the method has satisfied sensitivity. Compared with the first injection, the 660th chromatogram maintains well peak shape without tailing effect, and the shift of retention time is within 2%. The quality control after 660 samples present well accuracy within 80 to 120%.

**Conclusions**

The method offers an alternative to conventional sample preparation approaches. It requires low volume of urine samples (100 µL). Simple dilute-and-shoot successfully decreases sample preparation time and procedure. It provides a desirable method for high throughput analysis.
Abstract ID 274
Development of Thermo Scientific™ DRITM Zolpidem Enzyme Immunoassay for the Detection of Zolpidem and Its Major Metabolite Zolpidem Phenyl-4-COOH in Human Urine.

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Aim
Zolpidem, sold under trade names such as Ambien™ and Stilnoct™, is a schedule IV drug used to treat sleep disorders. It is an ideal insomnia drug because it has a quick onset with minimal residual daytime effects. Zolpidem is metabolized rapidly into Zolpidem Phenyl-4-COOH and Zolpidem 6-COOH, with only 1% of parent drug excreted in the urine. Zolpidem Phenyl-4-COOH accounts for >50% of all metabolites excreted in the urine, while Zolpidem 6-COOH comprises 11% of all metabolites. Commercially available immunoassays detect only zolpidem, but not its metabolites, which reduces the window of detection.

The objective of this study was to develop a liquid ready-to-use homogeneous enzyme immunoassay that can detect zolpidem and its major metabolite(s) in urine using the Thermo Scientific DRI immunoassay technology. Further, the antibody will have minimal cross-reactivity to structurally similar drugs that have imidazopyridine base structure.

Methods
DRI technology is based on competition between a drug labeled with glucose-6-phosphate dehydrogenase (G6PDH), and free drug from the urine sample, for a fixed amount of specific antibody binding sites. In the absence of free drug from the sample, the specific antibody binds the drug labeled with G6PDH and causes a decrease in enzyme activity. This phenomenon creates a direct relationship between the drug concentration in urine and enzyme activity. The enzyme activity is determined spectrophotometrically at 340 nm by measuring the conversion of nicotinamide adenine dinucleotide (NAD) to NADH. The performance of the assay was evaluated on the Beckman Coulter AU680 analyzer.

The DRI Zolpidem assay uses a 20 ng/mL cutoff calibrator with controls at ± 50% of the cutoff. The reagents, calibrators, controls are liquid ready-to-use. Reagents are compliant with REACH guideline (Registration, Evaluation, Authorization and Restriction of Chemicals). Patient samples were obtained from pain management laboratories. The samples were analyzed by LC-MS/MS to determine the levels of Zolpidem and Zolpidem Phenyl-4-COOH. Method comparison, cross-reactivity, interference, precision, spike recovery, dilution linearity, on-board, accelerated and real time stability studies were performed to determine the overall performance of the assay.

Results
Using a 20 ng/mL cut-off calibrator, the selected monoclonal antibody is specific to zolpidem, with approximately 100% cross-reactivity to its major metabolite Zolpidem-Phenyl-4-COOH. It does not cross-react with Zolpidem 6-COOH, Zaleplon or Zopiclone. The antibody has no significant cross-reactivity to other imidazopyridine compounds, such as, JWH-018-N-hydroxypentyl metabolite, AB-PINACA, UR 144 pentanoic acid, benzodiazepines, or structurally unrelated compounds, such as, opioids, tricyclic antidepressants, phenobarbital, chlorpromazine, diphenhydramine and synthetic cathinones. Method comparison study using patient samples showed > 95% agreement between immunoassay and LC-MS/MS. Precision study using spiked samples at 25% increment and decrement from the cut-off value demonstrated that all samples below cut off were negative and all the samples above cut off were positive, in both qualitative and semi quantitative mode. In qualitative mode, within run and total run precision was < 2% CV for cutoff calibrator and controls. Total run precision in semi-quantitative mode was < 8% CV for cutoff calibrator and controls. There was no interference from the endogenous and exogenous compounds, as well as with urine samples with specific gravity ranging from 1.004 to 1.029. Dilution linearity study demonstrated linearity of sample recovery throughout the calibration range with correlation coefficient of R2 = 0.9955.

Discussion
The data on the DRI Zolpidem Assay indicates excellent specificity and sensitivity to Zolpidem and its major metabolite Zolpidem Phenyl-4-COOH, without any significant cross-reactivity to other commonly abused drugs.

Conclusions
The DRI Zolpidem Assay has excellent specificity and sensitivity to Zolpidem and its major metabolite Zolpidem Phenyl-4-COOH.

NOTE: The assay is currently in development and is not approved by the FDA.

Keywords: Zolpidem, Metabolites, Immunoassay (DRI), Liquid Ready to Use

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Abstract ID  275
Customer Evaluation of Thermo Scientific™ CEDIA™ Mitragynine (Kratom) Assay in Drug Courts and Reference Laboratories.
Poulomi Acharya
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Thermo Fisher Scientific

Aim
Opiate addiction has reached epidemic proportions in the United States and people have turned to natural alternatives for self-management of pain. One such alternative is the use of Kratom (Mitragyna speciosa), a tree indigenous to Southeast Asia. The main alkaloids in Kratom include mitragynine, speciogynine, speciociliatine, paynantheine, and 7-hydroxymitragynine. Studies have identified 3 major metabolites for mitragynine: 9-desmethylmitragynine, 16 carboxymitragynine, as well as 7-OH mitragynine. Kratom is commonly ingested by chewing or smoking Kratom leaves, or drinking tea brewed using Kratom leaves. In the United States, Kratom can be purchased in various forms, including capsules, powders, e-liquid, and chocolate bars. Kratom is currently not a controlled substance federally but is listed as a “drug of concern” by the US Drug Enforcement Administration. Kratom is banned by several cities, counties and states within the US.

We recently developed the CEDIA Mitragynine (Kratom) Assay to detect mitragynine, the main alkaloid of kratom, in human urine. The assay was provided to drug courts and reference laboratories for evaluation. The assay is for Criminal Justice and Forensic Use Only.

Methods
CEDIA technology is based on the bacterial enzyme β-Galactosidase which has been genetically engineered into two inactive fragments, Enzyme Acceptor (EA) and Enzyme Donor (ED). These fragments spontaneously re-associate to form an active enzyme. In the absence of analyte from the sample, the specific antibody binds the ED-drug conjugate causing a decrease in enzyme activity. The free drug in the sample will compete for the limited number of antibody binding sites, making the ED-drug conjugate available for complementation to form an active enzyme. This phenomenon creates a direct relationship between the drug concentration in urine and enzyme activity. The enzyme activity is then determined spectrophotometrically at 570 nm.

The CEDIA Mitragynine reagents are provided in lyophilized form, while the calibrators and controls are liquid ready-to-use. The assay uses 5 calibrators (0, 20, 50, 100 and 200 ng/mL), with 50 ng/mL as the cutoff calibrator. The controls are at ± 25% of the cutoff (37.5 and 62.5 ng/mL). The assay was provided to drug courts and reference laboratories, together with draft application parameters for Thermo Scientific™ Indiko™ and Beckman Coulter™ AU™ series automated analyzers.

Results
A total of 1417 patient samples were tested from 12 laboratories from 7 states, including Ohio, Alabama, California, Oklahoma, Maryland, Massachusetts and Florida.

Using 50 ng/mL cutoff, 49 samples screened positive and were sent for LC-MS/MS confirmation. Of these 49 samples, 42 were confirmed to contain > 50 ng/mL mitragynine. The remaining 7 samples contained varying concentrations of mitragynine, ranging from 5.75 ng/mL to 47 ng/mL.

Discussion
The Thermo Scientific CEDIA Mitragynine (Kratom) Assay was able to detect mitragynine in human urine accurately when tested in drug courts and reference laboratories, with no false positives observed from other commonly prescribed or abused drugs.

Conclusions
The CEDIA Mitragynine (Kratom) Assay was able to detect mitragynine in human urine accurately.

NOTE: The assay is for Criminal Justice and Forensic Use Only and is not approved by FDA. The assay is not approved for sale in Europe.

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Abstract ID 277
First detection of l-methamphetamine and l-amphetamine as metabolites of selegiline in urine in South Korea.
Ilchung Shin
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Aim
Selegiline is a selective irreversible monoamine oxidase B inhibitor and is used to treat symptoms of Parkinson's disease. Its metabolites consist of l-methamphetamine and l-amphetamine which are known as enantiomers of illicit drugs, d-methamphetamine and d-amphetamine. It is, therefore, important to distinguish between l isoforms and d isoforms because methamphetamine and amphetamine are a regulated drug in South Korea as a potent central nervous system stimulant.

It was reported to the police that a 52-year-old male who repaired handbags for his living, showed in a store abnormal behavior which seems under the influence of intoxication. The police performed an initial urine test by using a methamphetamine detection kit (AccuSign). It showed a positive methamphetamine result. Given the initial results, the police officer requested a further analysis of his urine and prescribed drugs.

Methods
To test the prescribed drugs, we used the gas chromatography/mass spectrometry (GC/MS). Insofar as the urine is concerned, we investigated the simultaneous detection of selegiline, desmethylselegiline, methamphetamine and amphetamine, by using the headspace-solid phase microextraction (HS-SPME)-GC/MS. In addition, we also conducted a chiral study of methamphetamine and amphetamine found in the patient’s urine by using a chiral derivatization agent, (R)-(-)-α-methoxy-α-(trifluoromethyl) phenylacetylchloride (R-MTPCl) from which we detected the diastereomers by GC/MS.

Results
We identified selegiline, amantadine, ropinirole, quetiapine and tofisopam in his prescribed drugs. In his urine, we detected selegiline and its metabolites, desmethylselegiline, methamphetamine and amphetamine by HS-SPME-GC/MS. Consistent with previous studies, our results indicated that the ratio of amphetamine to methamphetamine was 0.32 which was in the range of selegiline administration, which was distinguished from methamphetamine abusers’ ratio of amphetamine to methamphetamine (mostly less than 0.24 in the previous results). In this chiral study, we successfully separated the diastereomers and thus confirmed l-methamphetamine and l-amphetamine as the metabolites of selegiline in his urine.

Discussion
Conclusions
This was the first case of having detected l-methamphetamine and l-amphetamine as metabolites of selegiline in South Korea. In this study, we first recognized that he had taken selegiline from the results of testing his prescribed drugs. We then investigated selegiline and its metabolites by HS-SPME-GC/MS in his urine and confirmed l-forms of methamphetamine and amphetamine by GC/MS after derivatization with R-MTPCl.
Abstract ID 280
Simultaneous determination of 21 synthetic cathinones in hair using liquid chromatography tandem mass spectrometry.
Hsiu Chuan Chen

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Chung Shan Medical University

Aim
In the past decade, the new psychoactive substances (NPS) of synthetic cathinones are abused and cause serious problems worldwide. The synthetic cathinones commonly known as "bath salts", can easily design to unregulated status by the exchanged small part of chemical structure but still with the same or enhance pharmacological activity. Because of low cost and easy accessibility via the Internet and head shops, these legal highs were to circumvent existing laws on controlled substances. In Taiwan, those drugs often mixed in 3 in 1 instant coffee bag, associated with other prevalent drugs, caused many criminal and death intoxications. The determination of synthetic cathinones is important for forensic and clinical toxicologists. There are some analytical methods were developed for the identification of synthetic cathinones in blood and urine samples, but the litter of hair testing. Hair had received obviously considerable alternative specimen because of its longer detection window (months to years). Specimen collection is non-invasive. It can provide a retrospective calendar of an individual's drug use by segmental analysis according to the length of hair. In this study, a reliable and sensitive analytical method of hair testing was developed for monitoring the 21 synthetic cathinones in Taiwan.

Methods
In this study, an identification and quantification analytical method of hair was developed for monitoring the 21 synthetic cathinones international, include methylcathinone (MC), fluoromethcathinone (FMC), chloromethcathinone (CMC), bromomethcathinone (BrMC), mephedrone (MM), 4-fluoro-α-PVP, 4-fluoro-α-PVP (FPVP), MPHP, 4-Chloro-α-PVP (CPVP), α-PHP (PV8), MDPB, MDPV. All of the authentic hair samples were decontaminated with dichloromethane, and then cut into small pieces and weighted 10 mg placed into glass tube. Hair was incubated with incubation media overnight at 25°C. The mass spectrometer was operated in positive electrospray ionization using multiple reaction monitoring (MRM) mode. The chromatographic reverse-phase separation was achieved with an Kinetex (50 mm x 4.6 mm) column packed with 2.6 μm core – shell C18 particles (Phenomenex, Torrance, CA, USA) of 0.1 % formic acid in water and in methanol. The total run time was 11 minutes.

Results
The results provide the limit of quantification (LOQ) < 6 pg/mg for most synthetic cathinones, except MEC, α-PVP. The r² of calibration curves were better than 0.99. Intra-assay and inter-assay imprecision were < 20%, respectively. Owing to verify the practicability of the developed method for monitoring 21 international common synthetic cathinones in hair, the LC–MS/MS method has been applied to analyze 90 hair samples from 2018 that originally tested positive for ketamine in our forensic laboratory. In our study, MM could be detected in 29 cases and concentration range is 3.3-1646.3 pg/mg. ML was found in 20 cases and concentration range is 4.2-1217.6 pg/mg. MC could be detected in 10 cases and concentration range is 13.2-193.9 pg/mg. BL was found in 8 cases and concentration range is 15-280.7 pg/mg. EMC could be detected in 5 cases and concentration range is 8.9-50.2 pg/mg. PL and CPVP were found in 3 cases and concentration range is 32.7-275.5 and 6.1-161.3 pg/mg, respectively. CMC was detected in 1 cases and concentration range is 10.8 pg/mg. α-PVP was detected in 1 cases but lower than LOQ. FMC, BrMC, MEC, DMMC, MD, FPVP, BzMC, MPHP, PV8, MDPB, MDPV was not found.

Discussion
The determination of synthetic cathinones is important for forensic and clinical toxicologists. Prevalence studies are hard to studies. One problem with synthetic cathinones is they do not have the common immunoassays and therefore cannot be detected by these screening tests. In addition, synthetic cathinones are often not routine analysis for the urine drugs testing laboratories. Therefore, a high estimated number of unreported cases can be assumed. Hair analysis is a useful tool for retrospective prevalence studies. It provides an overview of the drug consumption behaviour over several months. In this study, a reliable and sensitive analytical method of hair was developed for monitoring the common synthetic cathinones international.

Conclusions
We developed a sensitive and selective LC-MS/MS method for the simultaneous determination of 21 synthetic cathinones in hair. Only 10 mg specimens were required, synthetic cathinones were quantified LC-MS/MS. Chromatograms of an extract of drug-free hair sample spiked with standards was separation in 11 min. The LOQ was 3-12 pg/mg. The linearity was achieved in calibration curve coefficients (R²) were all above 0.99, and the precision and accuracy were less than 20%. The matrix effects for most synthetic cathinones were <35%. The method has been successfully applied to reanalyze 90 authentic hair specimens from forensic cases in 2018. The results show positive case and the order was MM > ML > MC > BL > EMC > CPVP = PL > CMC = α-PVP. This method provides a comprehensive confirmation method for 21 synthetic cathinones in hair, with good sensitivity and selectivity.
Abstract ID 282
Cytotoxicity of cationic disinfectants through apoptosis in human alveolar epithelial cells.

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Aim
Quaternary ammonium compounds (QACs) such as benzalkonium chloride (BAC) and cetylpyridinium chloride (CPC) are cationic surfactants and have been widely used as disinfectants, preservatives, and detergents for personal hygiene and medical use. In recent years, the growing use of QACs in a variety of applications has increased the risk of accidental and/or chronic inhalation exposure to QACs. It has been reported that inhalation of aerosols of BAC and CPC caused pulmonary damage in rats and mice. Therefore, inhaled BAC and CPC aerosols can adversely affect the cells in the alveolar region. In this study, we investigated the mechanism of cytotoxic effects of BAC and CPC using A549, a human alveolar epithelial cell line. Furthermore, we investigated an effect of the alkyl chain length on the cytotoxic potency of BAC homologues.

Methods
To examine cytotoxicity, A549 cells were exposed to CPC (0-1 mM), BAC (0-1 mg/mL), BAC homologues (C12-, C14-, C16-BAC, 0-0.2 mM), and pyridinium chloride (PC, as a control, 0-1 mM) for 2 days. Cell viability was assessed by AlamarBlue™. To examine involvement of apoptosis, A549 cells were exposed to BAC (0.02, 0.04 mg/mL) or CPC (0.02, 0.04 mM) in the presence or absence of Z-DEVD-fmk, a caspase-3-inhibitor, or Z-VAD-fmk, a pan-caspase-inhibitor, for 0~4 hrs and the activity of caspase-3/7 was measured by chemiluminescence method. In a parallel experiment, the cells were lysed with RIPA lysis buffer and the activation of caspase-3 and cleavage of PARP were analyzed by western blotting.

Results
The viability of BAC- or CPC-exposed cells was decreased in a dose-dependent manner. The values of LC50 in BAC- and CPC-exposed cells were 0.009 mg/mL and 0.008 mM, respectively. The cytotoxicity was suppressed by N-acetylcysteine (NAC), a glutathione precursor. Furthermore, comparison of cytotoxicity among BAC homologues showed that C16-BAC was more cytotoxic than C12- or C14-BAC. Conversely, addition of PC did not show the cytotoxicity. Caspase-3/7 activity was increased in BAC- or CPC-exposed cells, and the activity was abolished by caspase-3- and pan-caspase-inhibitor. Western blotting revealed that the cleaved active form of caspase-3 and cleaved PARP fragment were detected in BAC- or CPC-exposed cells.

Discussion
The cytotoxicity increased by BAC and CPC dose-dependently was suppressed by NAC, suggesting that the cytotoxicity was related at least in part with reactive oxidative species (ROS) generation. Furthermore, cytotoxicity assay revealed C16-BAC, which has the longest alkyl chain, was more cytotoxic than C12- or C14-BAC, while PC, which has no alkyl chain, did not show the cytotoxicity. These results suggest that cytotoxicity of BAC and CPC might be due to long alkyl chain. Caspase-3/7 activity and cleaved active form of caspase-3 and cleaved PARP fragment were increased in BAC- and CPC-exposed cells. These results indicate that BAC and CPC cause cell death via caspase-3-dependent apoptotic pathway in A549 cells.

Conclusions
Cytotoxic potency induced by BAC and CPC in A549 cells might depend on the long alkyl chain. Moreover, this study revealed that BAC and CPC cause cell death via caspase-3-dependent apoptotic pathway in A549 cells.
Abstract ID 284
Comparison of in vitro and in vivo models for elucidation of metabolic patterns of 7-azaindole-derived synthetic cannabinoids exemplified using CUMYL-5F-P7AICA.

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Aim
Since their appearance on the drugs of abuse market, synthetic cannabinoids (SC) are gaining increasing forensic toxicological relevance and have become a tremendous public health concern. Due to the highly dynamic market concerning the emergence of new SC, the determination of analytical targets is often challenging in the field of clinical and forensic toxicology. As these substances are extensively metabolized, their main metabolites have to be known for a sufficient detection in biological matrices, particularly in urine. However, SC are sold and consumed without the knowledge of their toxicokinetic and toxicodynamic properties, because results of controlled human studies are usually not available. Thus, the aim of the present work was to elucidate the general in vitro and in vivo phase I and II pathways of 7-azaindole-derived synthetic cannabinoids by using 1-(5-fluorpentyl)-N-(2-phenylpropan-2-yl)-1H-pyrrolo[2,3-b]pyridin-3-carboxamide (CUMYL-5F-P7AICA) as example. Different metabolizing systems should be compared and monoxygenase isoforms responsible for the major metabolic steps should be identified.

Methods
Pooled human S9 fraction (phS9, 2 mg/mL) were incubated with CUMYL-5F-P7AICA (25 µM) for one and six hours after addition of co-substrates necessary for common phase I and II reactions. Human liver microsomes (HLM, 1 mg/mL) and pig liver microsomes (PLM, 1 mg/mL) were incubated for 30 min with the substrate at 25 µM following the addition of co-substrates for common phase I reactions. Acetonitrile was used for termination of reactions. Rat urine was collected over a period of 24 h after oral administration of a single dose of CUMYL-5F-P7AICA (0.6 mg/kg) for toxicological diagnostic purposes. Sample preparation of urine samples consisted of precipitation by acetonitrile with or without prior enzymatic conjugate cleavage. All samples were analyzed applying high resolution MS/MS (positive mode) following reversed-phase LC. Incubations with recombinant human monoxygenases were performed according to previous studies (Caspar et al., ABC, 2018).

Results
The parent compound could still be detected in high abundances in all models except for the in vivo model rat. Only small amounts of the parent compound were excreted into rat urine. Common major metabolic steps in the three in vitro models were oxidative defluorination followed by carboxylation, monohydroxylation and ketone formation. Regarding phS9, oxidative defluorination as well as monohydroxylation followed by sulfation or glucuronidation were determined as additional main metabolic steps. HLM and PLM additionally formed high abundant N-dealkylated and dihydroxylated metabolites. The major metabolic steps in vivo were oxidative defluorination, carboxamide hydrolysis and oxidative defluorination followed by carboxylation, N-dealkylation, N-dealkylation followed by hydroxylation, and dihydroxylation. In general, less metabolites could be determined in the in vivo specimens as compared to the in vitro samples. The defluorinated as well as monohydroxylated (and sulfated/glucuronidated) and dihydroxylated metabolites can be recommended as urinary screening targets besides the parent compound. Initial monoxygenase activity screening revealed the involvement of CYP1A2, 2C8, 2C9, 2C19, 2D6, 3A4, and 3A5 in the initial hydroxylation. Regarding the formation of dihydroxy metabolites, CYP2C19, 3A4, and 3A5 were involved. Oxidative defluorination was catalyzed by CYP2C8, 2C9, 2C19, 2D6, and 3A5.

Discussion
In comparison with already published metabolism studies (e.g. Staeheli et al. FSI 2019), the present investigation covered phase I as well as phase II metabolites following administration under controlled conditions. Monoxygenases involved in the initial metabolic reactions were additionally identified. The results were in good agreement with data previously found in HLM incubations and authentic human urine specimens. Contrarily to most of the synthetic cannabinoids, CUMYL-5F-P7AICA is also excreted into urine as non-metabolized parent compound. Furthermore, it does not undergo substantial phase II biotransformation and metabolites formed by HLM and PLM were comparable. Less metabolites were found in vivo as compared to in vitro. The recommended urinary targets should be used to prove an intake in human urine specimens.

Conclusions
The present study demonstrated that CUMYL-5F-P7AICA in vivo is not as extensively metabolized than in vitro. However, similar main phase I metabolites were found in vivo as compared to in vitro. The tested in vitro and in vivo models are suitable for the elucidation of metabolic patterns of 7-azaindole derivatives of the SC.
Abstract ID 297

A simple and fast method for busulfan quantification by UPLC-ESI-MS/MS and application for therapeutic monitoring in haematopoietic stem cell transplantation.

Susana Simões

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Aim

The purpose of the present study was to develop and validate an ultra performance liquid chromatography tandem mass spectrometry method that allows the quantification of busulfan in plasma samples. Intravenous Busulfan is an alkylating drug routinely used in conditioning regimens before allogeneic haematopoietic stem cell transplantation (HSCT), the standard treatment for various malignant and non-malignant disorders. Busulfan shows a wide intra- and interindividual variability in pharmacokinetics and a narrow therapeutic window. High exposure (expressed as area under the curve [AUC]) is associated with an increased risk of toxicity, such as mucositis, graft-versus-host disease (GvHD), and venoocclusive disease (VOD) or sinusoidal obstructive syndrome, and transplantation-related mortality. A low busulfan AUC has been associated with a higher probability of graft rejection or disease relapse. As a result, many centers have attempted to personalize the dose of busulfan using Therapeutic Drug Monitoring (TDM) which has been associated with important clinical outcomes in these patients.

In Portugal the busulfan TDM program was implemented in 2014. This procedure is pioneer at a national level and it relies on a strict protocol which includes collaboration with several hospital departments of the Portuguese Institute of Oncology and the Laboratory of Forensic and Chemical Toxicology of the National Institute of Legal Medicine and Forensic Sciences, where the analytical procedure was developed and fully validated.

Methods

The plasma samples were cryopreserved at -80°C until analysis by using a simple protein precipitation followed by a fast ultra-performance liquid chromatographic with electrospray ionization tandem mass spectrometry (UPLC-ESI-MS/MS) method, using positive ionization and multiple reaction monitoring mode (MRM). Deuterated busulfan was used as the internal standard. The chromatographic separation was performed with an Acquity UPLC® HSS T3 (100 x 2.1 mm i.d., 1.8 µm) reversed-phase column using a methanol/2 mM ammonium formate buffer pH 3.4 gradient and the MS/MS detection was achieved with two precursor-product ion transitions. The method was validated according to international guidelines. Individual PK parameters values (clearance and volume of distribution) for a one compartment model were estimated using a maximum likelihood estimation modelling algorithm included in ADAPT 5.0 software. The AUC per busulfan dose and dose adjustments were calculated using Excel.

Results

The UPLC-ESI-MS/MS method presented high accuracy (<5%), within-run and within-laboratory precision with CV ≤ 6%. Recoveries were within the range of 89-104%. Linearity was verified from 0 to 8,000 ng/mL (r² >0.99). No significant matrix or carryover effects were observed. Limit of detection and limit of quantification were 20 and 50 ng/mL, respectively. The validated method was applied to measure busulfan levels from 51 patients (with ages between 10 months and 63 years old; 18 females, 33 males; 41 oncological diseases, 10 non-oncological diseases), 7 of them were included in a trial phase without dose adjustments.

Discussion

A reliable method was developed to personalize the dose of busulfan using TDM, which has the potential to improve transplant outcomes. Regarding busulfan dose adjustment in the multiple dose regimen (16 patients), the dose was changed in 94% of the patients, with a maximum increase of 25% and a maximum decrease of 37% (with a median value of -8%). Regarding busulfan dose adjustment in the once daily dose regimen (28 patients), the dose was changed in 89% of the patients, with a maximum increase of 29% and a maximum decrease of 38% (with a median value of -1%). The impact of TDM during 3 days versus the expected AUC obtained without TDM (using the body-weight recommendations) on 27 patients (10 patients in the multiple dose regimen and 17 patients in the once daily dose regimen) was also evaluated. TDM has statistically significant advantage over the BW recommendations. Concerning the multiple dose regimen, 70% of subjects within the acceptable 10% difference to target AUC, against 30% on the BW approach and in the once daily dose regimen 88% of subjects within the acceptable 10% difference to target AUC, against 35% on the BW approach. Clinical outcomes evaluation is ongoing. No VOD in this cohort of patients occurred.

Conclusions

The results obtained so far indicate that this tool, which can be used for other medicinal products, represents a huge benefit for patients and may represent a reduction in the costs associated with the treatment of problems related to toxicity.
Abstract ID 301
Uncovering Tracks – Robust, Reproducible Screening Assay for Fentanyls in Urine with LC-HRAM(MS).

Magnus Olin
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Aim
Fentanyl is an opioid used as a pain medication together with other medications for anesthesia. Fentanyl and fentanyl analogues made illegally are also used as recreational drugs. Fentanyl and its analogues are significantly stronger than morphine, with some analogues (carfentanil) exhibiting ~10,000 times higher strength than regular pain medications. The use and abuse of fentanyl and its analogues are also known to cause serious side effects, ranging from respiratory depression to deaths. Problems related to fentanyl and its analogues are still prevalent in many countries according to reports from European Monitoring Centre for Drugs and Drug addiction (EMCDDA). In 2017, deaths due to overdoses of fentanyl and fentanyl analogues were more common than deaths owing to heroin overdose in Sweden, (data from Swedish National Board of Forensics). To detect fentanyl and some of the common fentanyl analogues in urine, we developed a screening method using LC-HRAM.

Methods
A Screening method based on reversed phase liquid chromatography (LC) coupled to High Resolution Accurate Mass (HRAM) spectrometry for 14 different Fentanyl analogues and metabolites in urine has been developed using a Thermo Scientific™ Ultimate RS3000 UHPLC system and a Q Exactive Focus™ Orbitrap™ High Resolution Accurate Mass spectrometer operated in data dependent MS/MS (ddMS2) mode. Identification was based on m/z and retention time. Confirmation of the compounds was performed by matching of the MS2 spectrum of the target compound to the recorded library MS2 spectrum. Ion chromatograms were extracted at ± 5 ppm and quantitation was based on a two point calibration curve using internal standard calibration. 4 different fentanyls labeled with stable isotopes were used as internal standards. The total analysis time was 5 minutes. Sample preparation was performed by dilution and direct injection of urine into LC-HRAM(MS).

Results
The method developed in this study was tested and validated by monitoring accuracy of identification, linearity, matrix effects, accuracy and Coefficient of Variation (%CV) at a concentration close to the cutoff. All compounds present in the spiked matrix were detected and confirmed at the lowest level required for these compounds, 0.5 – 5 ng/mL. Quantitation was performed to determine if detected concentrations were above the cutoff value of the method. The accuracy and %CV for every analysis met the requirement of ±15% for all compounds. The identity of all compounds was confirmed by the accurate mass (±5 ppm), the retention time (± 0.25 min) and matching against a spectral library (reversed search) recorded using pure standards.

Clinical urine samples were screened for fentanyl and fentanyl analogues. Only small portion of the tested samples were positive for fentanyl and no fentanyl analogues were present. No false positive samples were detected in the screened urine samples.

Discussion
The detection window for fentanyl is short. Hence, the positive rate of fentanyl and fentanyl analogues screened in urine samples were very low. Fentanyl concentration in urine samples is present at a low level. Therefore, screening fentanyl and its analogues using LC-MS method is advantageous over immunoassay methods which needs a higher cut-offs. There is a steady increase in use and demand of implementation of methods for known/unknown screening and untargeted/targeted quantitation of analytes in complex biological matrices. Methods based on Thermo Scientific™ QExactive™ are suitable for this task since data can be collected in untargeted mode, which makes gives the possibility for retrospective interrogation of data, to investigate the presence of e.g. new internet drugs. The instrument is sensitive enough for quantitation, even at the low levels required for Fentanyls, and it can provide HRAM spectra that together with retention time and accurate mass provides unambiguous identification.

Conclusions
In this study, we report a robust, reliable, and reproducible method for screening of Fentanyls in Urine.
Abstract ID 309
Confident Quantitation with LC-MS/MS: Fast, Robust, Reliable, Reproducible, Sensitive Quantitation of Drugs of Abuse in Urine.

Debadeep Bhattacharyya

Debadeep Bhattacharyya1, Kevin McHale2
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Aim
Aim: Development and implementation of a robust, reliable, reproducible, and sensitive workflow for analysis and quantitation of several drugs of abuse in urine using liquid chromatography coupled with triple quadrupole mass spectrometry.

One of the major challenges for analytical laboratories monitoring drugs of abuse in biological matrices is analyzing hundreds of samples every day while addressing the significant challenge of catching all the attempts that are made to bypass controlled substance laws, as well as identifying and quantifying novel compounds that are appearing in the market. In the last few years, multiple quantitation technologies have been developed to address this challenge. However, liquid chromatography (LC) coupled to mass spectrometry (MS) has gained widespread popularity owing to its inherent ability to offer increased selectivity, specificity, robustness, and sensitivity. In this report, we investigate the feasibility of high-throughput measurements of 53 drugs of abuse and metabolites in forensic toxicology by reducing time-consuming sample preparation steps and employing two-minute UHPLC-MS/MS analyses per sample.

Methods
A fast, robust, reliable, reproducible method for targeted screening and quantitation was developed based on reversed phase chromatography (LC) coupled to TSQ Quantis triple-stage quadrupole mass spectrometry (MS) for confirmation and quantitation of 53 drugs of abuse and their isomers in urine samples. LC separation was accomplished with the Thermo Scientific™ Vanquish™ Horizon UHPLC system using a binary reverse-phase gradient running with a flow rate of 1.0 mL/min. A Thermo Scientific™ TSQ Quantis™ triple-stage quadrupole mass spectrometer6 was used for acquiring MS data. All compounds for this study were analyzed in positive ion mode with a total of 210 SRM transitions. All data were acquired, processed, and reviewed using Thermo Scientific™ TraceFinder™ 4.1 software.

Results
More than 75 drugs of abuse and their metabolites were analyzed and quantified with one LC-MS/MS method having an acquisition time of less than 1.4 min. The method ensured high productivity that helped achieve critical throughput goals demanded by most analytical laboratories. Most target compounds had LLOQs at or below the designated cutoff levels in diluted urine. There are several overlaps between elution times of different analytes, which makes separation between isomers and also separation between analytes challenging. However, additional selectivity and speed offered by the TSQ Quantis instrument enabled the ability to address these challenges in this study.

Discussion
Separation of isomers: At 1 mL/min with a 1.9 μm particle column, the observed LC peak widths were typically about ~1.3 s at the base. The LC flow rate is with no split to the TSQ Quantis HESI source. Opiate isomers have the same precursor m/z and many generate the same product ions. Hence, it is necessary to chromatographically separate these compounds. Four opiate isomers that have a molecular weight of 286 are easily separated by UHPLC at 1 mL/min, and the TSQ Quantis instrument has sufficient acquisition speed to accurately quantify these compounds. Multiple SRMs were needed for some of the analytes, especially those with isomers, to ensure ideal separation within the required run time. Separation of analytes: The acquisition speed and detection efficiency of the TSQ Quantis instrument are critical in such situations, especially for narrow LC peaks. The elution of 6-MAM and buprenorphine occur at the times of higher numbers of SRM transitions, hence, with the lowest duty cycles and dwell times. With a fast UHPLC method, analytes like 6-MAM and buprenorphine elute with many other compounds. The increased number of SRM transitions with low SRM dwell times enables increased productivity. The acquisition speed and detection efficiency of the TSQ Quantis MS are critical in such situations, especially for narrow LC peaks. With a fast UHPLC method, 6-MAM and buprenorphine elute with many other compounds, which requires the flexibility of a short dwell time.

Conclusions
Analysis and quantitation of drugs of abuse in biological matrices can pose several challenges, especially with the growing demands of increasing throughput and high sensitivity. LC-MS/MS with liquid chromatography and triple quadrupole mass spectrometry offers several advantages in performing robust, reproducible, fast, and sensitive quantitation of drugs of abuse across several biological matrices, especially urine. In this study, we demonstrate the highly reproducible chromatographic performance of the Vanquish Horizon UPHLC system along with the outstanding speed and sensitivity of the TSQ Quantis mass spectrometer to perform confident quantitation of several drugs of abuse and metabolites in diluted urine for forensic toxicology samples in ~2 minutes per sample.
Abstract ID 316
Detection of cocaine in capillary blood: adhesion or consumption?
Lars Wilhelm
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LADR MVZ Dr. Kramer und Kollegen

Aim
Although urine is the most common matrix in clinical toxicology, in case of pre-analytical, pharmacological as well as ethical considerations other matrices like oral fluid or blood became more important in the last years. Because of the bad vein status of i.v. drug users capillary blood is a promising opportunity. An unexpected observation of capillary blood is the fact that besides its metabolites also the parent drug is frequently found, especially for cocaine. Considering the short elimination half time and the minor stability caused by hydrolysis in blood samples the parent drug is quickly excreted. Aim of this study was to investigate the concentration of cocaine and its metabolite benzoylecgonine in capillary blood as a hint for adhesion as a potential source of the parent drug and the implementation for the assessment in clinical toxicological examinations.

Methods
The sample preparation of the capillary blood was performed by a solid phase extraction with a weak cation exchanger phase. Chromatographic separation is done by biphenyl phase. For mass spectrometric detection two transitions in MRM mode were used. As internal standard benzoylecgonine-d3 was used. The mass spectrometer was an API6500Q. The method was fully validated for cocaine and its metabolite benzoylecgonine. Samples were selected from clinical drug testing with end to end capillaries. The data where evaluated for the concentration of benzoylecgonine and the appearance of cocaine. Statistical analysis using the statistical program R was performed by anonymous evaluation.

Results
8743 datasets were examined in this study. 754 were included for the examination. In 8.6% of the samples cocaine or its metabolite benzoylecgonine were detectable. In 619 (82%) datasets only benzoylecgonine was found. 135 samples (18%) were both positive for benzoylecgonine and cocaine. For further informations from these data we analysed the benzoylecgonin concentration of two different groups. Group A was defined for only benzoylecgonine positive samples. In group B the samples positive for benzoylecgonine and cocaine were included. The interquantile range (IQR) of the boxplot was much smaller in group A than for group B with positive results. The quantil of group A is ranging from 26 – 230 ng/ml. Group B shows a range from 277 – 1200 ng/ml. This results are statistical significant (p-value < 0,01).

Discussion
Our results show that in samples in which cocaine as parent drug is found the concentration of benzoylecgonine is higher, than in samples without cocaine. A possible explanation for this could be adhesion. Interpretations should always consider the risk of possible contamination of the blood sample. During sample taking adhesions on the skin can cause this effect. Because of the ex-vivo hydrolysis of cocaine a body passage cannot be proved by findings of benzoylecgonine. A metabolite not formed by hydrolysis should be added to LC-MS/MS method to detect contaminations of the sample.

Conclusions
The presented data give a suspicion that adhesion play a role in drug testing from capillary blood samples. The metabolite ratio cannot prove this. Further studies and investigations in clinical cases could be done by analysis of the swab for disinfection to detect possible adhesions on the skin. Studies in venous blood should be done to assess these results.
**Abstract ID 347**

**11-OH-THC in hair as marker of active cannabis consumption: estimating a reliable cut-off by evaluation of 672 cannabis users.**

**Sara Casati**

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**Aim**

Discrimination of cannabis active use from passive consumption by hair analysis may fail when performed by the sole detection of compounds present in plant material as well as in cannabis smoke such as Δ9-tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN). For this reason, the determination of THC metabolite 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH) has been proposed by the Society of Hair Testing (SoHT) in order to prove active cannabis consumption. THC-COOH is formed from THC via 11-hydroxy-Δ9-tetrahydrocannabinol (11-OH-THC) by cytochrome P450 enzymes. The identification of THC-COOH in hair is still challenging due to its acidic nature and the its critical low concentration, since the incorporation into hair shaft appears to be preferential for basic compounds. Alternatively, 11-OH-THC may be considered as a complementary marker for THC administration. Our recent study reported an accurate validated procedure for THC, CBD, CBN and 11-OH-THC in hair, based on a GC/MS-MS method in electron ionization mode. However, unlike THC-COOH, a cut-off level for 11-OH-THC in hair has not been fixed yet. Therefore, the aim of this study is to propose a concentration value for 11-OH-THC in hair analysis in order to discriminate between chronic and occasional use.

**Methods**

GC-MS/MS methods normally used in our laboratory to detect THC, 11-OH-THC and THC-COOH were applied to 672 hair samples from cannabis users tested for drugs chronic abuse in our Laboratory from 2015 to 2019. The Receiver Operating Characteristics (ROC) analysis was applied for cut-off evaluation after 11-OH-THC (test method) and THC-COOH (control method) quantification. The comparison of the results for THC-COOH and 11-OH-THC was also made by means of the Cohen’s kappa statistics in order to evaluate their agreement according to the Landis and Koch scales.

**Results**

A total of 672 cannabis users (THC > 0.01 ng/mg) were included in the calculations, THC-COOH and 11-OH-THC have been quantified (> LLOQ) in 513 and 578 hair and in 73 and 81 hair samples, respectively. The calculated mean values of the two metabolites concentrations were considerably different: the mean values found in the THC-COOH group (0.82 ng/pg) were 30% lower compared to the group of 11-OH-THC (1.17 ng/pg) in hair, whilst 43% higher in body hair samples (THC-COOH mean value 3.03 ng/pg ; 11-OH-THC mean value 1.72 ng/pg). The ROC test was performed considering a cut-off equal to 0.01 pg/mg for THC-COOH (test method). The ROC curve AUCs for 11-OH-THC were 0.874 with a standard error (SE) of 0.01 and 95% confidence interval (CI) ranging from 0.845 to 0.900 (p value <0.001) for 590 hair samples and 0.879 with a SE of 0.05 and 95% CI ranging from 0.789 to 0.941 (p value <0.001) for 82 body hair samples. That means that 87% and 84% of positives ranked before a uniformly drawn random negative. For determining the optimal cut-off, a minimum sensitivity and specificity of 80% was defined. The Youden’s index suggests a cut-off value of 0.51 pg/mg in hair and 0.52 pg/mg in body hair specimens. By considering a threshold for a sample to be considered positive equal to 0.2 pg/mg for THC-COOH and for 11-OH-THC equal to 0.51 pg/mg in hair and 0.52 pg/mg in body hair specimens, in a total of 590 hair and 82 body hair samples evaluated the Cohen’s Kappa statistics was 0.65 and 0.66, respectively.

**Discussion**

Higher cannabinoids concentrations have been found in body hair versus scalp hair. These differences are explained by increased incorporation from sweat or sebum during the longer telogen stage, representation of another time period because of the different growth cycle, differences in pigmentation and less exposition to light, weather and cosmetic treatments. However, the mean values relative to 11-OH-THC seem to be more reproducible than THC-COOH in the two groups considered. Moreover, to see if the results might be biased correlated, the Pearson product-moment correlation coefficient between THC-COOH and 11-OH-THC concentrations was calculated showing a slightly low correlation coefficient of 0.25. In parallel, median concentration ratios of THC to 11-OH-THC and THC to THC-COOH of 0.56% and 0.31% for positive hair samples were found and may serve as an indicator of chronic abuse of cannabinoids. For the proposed cut-off values equal to 0.51 pg/mg in hair and 0.52 pg/mg in body hair specimens, we found a sensitivity and specificity of 80.2 and 85.9 % for hair samples, 89.1 and 88.9 for body hair samples, respectively. Moreover, the comparison of the results for THC-COOH and 11-OH-THC made by means of the Cohen’s kappa statistics providing a good agreement according to both Landis & Koch and Fleiss scales.

**Conclusions**

In conclusion, we suggest that the quantification of either THC-COOH or 11-OH-THC (> 0.5 pg/mg) should be mandatory in order to prove active intake and exclude false positive result from external contamination.
Abstract ID 350
The effect of storage time on the stability of FAEE in hair samples: results from a pilot study.
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Aim
The advantages of analysis of drugs in hair samples are recognised for the long window of detection, alongside easy sampling and long stability after sample collection. Whilst stability of drugs in hair samples is well documented there is no equivalent data available for the alcohol markers. The main objective of this pilot study was to investigate stability of hair samples kept in storage to determine whether it had a detrimental effect on the stability of the fatty acid ethyl esters (FAEE) through time.

Methods
Intact hair samples that had been originally tested for total FAEE at various times were randomly selected when there was sufficient remainder hair for a re-test. All hair samples (N=24) were stored wrapped in aluminium foil which were enclosed in paper envelopes and were stored at room temperature in dry and dark conditions between 4 days and 80 months. All re-tested hair sections represented the same segment lengths for equivalent time of the first tested periods. The first test and the re-test comprised the analysis of the four major FAEEs: ethyl myristate, ethyl oleate, ethyl palmitate and ethyl stearate, using the same methodology by LC-MS/MS utilising a validated in-house method which was accredited to ISO/IEC 17025 standards. Total FAEE and in particular Ethyl Palmitate (EtPal) were statistically evaluated. Paired-sample T-test was conducted to compare the results of the first test with the results of the second test (p=0.005).

Results
The statistical analysis of the data showed a significant difference of total FAEE levels between the first test (M=7372.7, SD=5747.7) and the second test (M=1136.8, SD=2109.2 pg/mg of hair); t(23)=5.04, p < 0.005. When the results were grouped according to storage time, hair samples re-tested within 1 month from collection showed decreased levels of total FAEE, on average 38% (from M=3860.0, SD=3067.7 to M=2364.7, SD=2872.9 pg/mg of hair). When samples were stored for periods over 1 month and up to 80 months, total FAEE levels decreased on average 100% (from M=9881.7, SD=5971.8 to M=259.7, SD=410.7 pg/mg of hair), with some samples showing no detected levels of FAEE. Similar pattern of results was obtained when focusing on the EtPal data which showed an equally significant difference between the first test (M=3620.4, SD=2447.8 pg/mg of hair) and the second test (M=602.8, SD=1044.0 pg/mg of hair); t(23)=5.75, p < 0.005. Hair samples re-tested within 1 month from collection day showed decreased levels of EtPal, on average 50% (from M=2285.2, SD=1719.1 to M=1217, SD=1430.9 pg/mg of hair). When samples were stored for periods over 1 month and up to 80 months, EtPal levels decreased on average 100% (from M=4574.1, SD=2490.4 to M=164.1, SD=15 pg/mg of hair), with some samples showing no detected levels of EtPal.

Discussion
The sum of the four esters of FAEE levels or the levels of EtPal in isolation measured on the same hair length over the same time period segment showed significantly lower levels after being stored over a relatively long time. It is not clear what causes the deterioration in hair samples, but at the moment we can speculate that EtPal had decomposed or evaporated. Nonetheless, the study data suggested that total FAEE in hair samples deteriorates significantly even when stored under ideal conditions. Specifically, our results suggest that when measuring total FAEE or solely EtPal in hair samples, the elapsed time between sample collection and analysis of the sample needs to be considered when interpreting the results.

Conclusions
The recommendation remains that whether hair samples need to be tested for total FAEE or EtPal, the analytical procedure needs to be performed shortly after collection in order to obtain meaningful results.
Abstract ID 369
Identification of Disulfiram-like Reaction in Forensic
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Aim
Disulfiram-like reaction, an acute poisoning for acetaldehyde accumulation in vivo, is caused by alcohol consumption after antibiotics intake. The symptoms include facial flushing, headache, abdominal pain, sweating, palpitations and breathing difficulties, even lost consciousness or death in serious cases. In China, forensic cases involving disulfiram-like reactions are common, due to the abuse of antibiotics, especially cefoperazone. However, there is no exact criterion to determine a death caused by disulfiram-like reaction. In order to provide forensic evidence for related medical disputes and death cases after drinking, it is necessary to establish a method for identification of disulfiram-like reaction. In this study, we developed a method and verified its feasibility by animal models. For further study, this method should be validated and applied to clinic for diagnosis.

Methods
For animal experiments, SD rats weighting 200g were divided into three groups: control group A, control group B and an experimental group, with 6 rats in each group. The rats of control group A were injected intravenously with saline (0.9% NaCl) at tail once a day for three consecutive days. After the last injection, they were orally administered alcohol beverage and killed by cervical dislocation one hour later. As for rats of control group B, cefoperazone was injected for three days like group A. After oral administration of saline on the third day, they were killed by the same way. The experimental group was treated by cefoperazone injection and oral administration of alcohol beverage. Venous blood samples were collected from their inferior vena cava. Acetaldehyde in blood was detected by headspace-gas chromatography (HS-GC). 0.5ml blood sample and 0.5ml 40 g/mL tert-butanol were added to 10mL headspace sample bottle, cover with aluminum cover and mix well, then put into headspace sample injector. Cefoperazone was detected by LC-MS/MS. 0.5ml blood sample was precipitated by 1mL chromatographic grade methanol. The mixture was centrifuged for 15min at 15,000rpm. 1µL supernatant was injected into LC-MS/MS for analysis. The obtained results of experimental group were compared with control group A and B respectively.

Results
The calibration curve for acetaldehyde was ranging from 5 to 200µg/mL and cefoperazone was ranging from 0.1 to 50µg/mL, each of which had a correlation coefficient (R2) > 0.99. The LLOQs of acetaldehyde and cefoperazone were 5µg/mL, 0.1µg/mL, respectively. The two control groups were detected Acetaldehyde negative. The blood acetaldehyde concentration of experimental-group rats was from 8.40 to 11.96µg/mL. Control group B was detected cefoperazone positive, as well as experimental group.

Discussion
Disulfiram-like reaction induced by cefoperazone was the most common in China. Therefore, this method is expected to be used in the identification of suspected cefoperazone-induced disulfiram-like reaction. However, we should also note that cefoperazone is metabolized rapidly and it is not the only medicine that can lead to disulfiram-like reactions. Therefore, we should continue to improve the method in the future to handle disulfiram-like reaction cases under different conditions. For further study, it could be established according to the actual situation that a rapid detection method for acetaldehyde dehydrogenase activity and a drug-screening method for determination of multiple common clinical medicine consumption, which lead to disulfiram-like reactions.

Conclusions
An accurate and rapid method for the determination of acetaldehyde and cefoperazone in blood was established and verified by animal experiments. The results of acetaldehyde detection showed that the experimental group SD rats had disulfiram-like reaction, which proved that the method could be used to detect the disulfiram-like reaction preliminarily, and acetaldehyde could be an objective index for clinical and forensic diagnosis of suspected disulfiram-like reaction.
Abstract ID  370
Ethyl glucuronide concentration in hair of detainees: a preliminary study.
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Aim
Through the measurement of EtG in hair (hEtG), it is possible to assess both the chronic alcohol abuse over time and to document the treatment efficacy of patients in a withdrawal program. Moreover, various situations necessitate total abstinence from ethanol, that can also be monitored through hETG; among them, jails constriction on the base of security and medical reasons. Notwithstanding the general prohibition, efforts are made by the inmates to obtain and consume all kind of illicit substances as well as ethanol. In this paper we present a preliminary study on hETG in inmates of an Italian prison in order to confirm or deny alcohol intake.

Methods
A voluntarily based and anonymous samples collection of hair samples was set on 102 inmates. All participants were above 18 years old and in order to avoid misinterpretation, no hair from other body district than head were collected. All samples were analyzed on proximal 3 cm segment by Agilent 1290 system coupled to an Agilent 6460 triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA). EtG was separated employing an InfinityLab Poroshell 120 HILIC-Z column (3.0 x 100 mm, 2.7 μm) and the analytes were detected in the multiple reaction monitoring mode (MRM), monitoring three transitions for EtG (m/z 221 → 75; 221 → 85; 221 → 113) and one transition for d5-EtG (m/z 226 → 85).

Results
Samples were from subjects born in Italy in 40% of cases, 16% were from Eastern Europe and 44% from Northern or Central Africa. EtG above LOQ was determined in 40%, 12% and 50% of Italian, East European and North/Central Africans, respectively. Female and male distribution was 28% and 72%, respectively. Average male age was 34 yo (19-55 yo), while average female age was 40 yo (22-55 yo). Ten percent of subjects declared regular alcohol consumption, while 66% denied any. Fourteen percent and 10% of test persons declared respectively occasional alcohol use or past-abuse behavior. The average detention period was 23 days, from a minimum of 2 up to 74 days.

ETG findings were classified in four concentration ranges: ETG < LOQ (86%); LOQ< ETG< 7 pg/mg (2%); 7 < ETG< 30 pg/mg (6%) and ETG > 30 pg/mg (6%). Positives (6%) were samples with concentrations ranging from mild (42 pg/mg) up to strong positiveness (270 pg/mg), while all other groups were considered negative (94%) according to SoHT recommendations.

Discussion
Though the small number of analysed samples, to the best of our knowledge, this represents the first preliminary study on ETG hair concentration on inmates in prison. In fact, due to the limited access to this special population, very rarely it is taken into consideration and very few papers are available in literature. In our opinion, the data collected with this preliminary study may be interesting 1) when compared to results originating from other kind of populations, not offenders and 2) when ETG findings are associated to longer detention period i.e. Does concentration of ETG in hair (i.e. alcohol consumption) increase or decrease during detention? this study shows a correlation between ETG concentration in hair and detention days. All positive samples are within 12 days, confirming an alcohol intake prior incarceration. However, some papers show how in older prisoners a significantly higher rates of major illnesses and functional impairment related to alcohol misuse exists and that alcohol abuse remains one of the health problems associated to detention, though alcohol is prohibited in jail.

Conclusions
The values obtained with this experimental preliminary study is interesting since most data present in literature on the consumption of alcohol in jail cover epidemiological studies based on self-reported questionnaires. Furthermore, the utility to perform toxicological screening on alcohol at the admittance to prison as well as during detention, may help in set a personalized clinical treatment and to avoid or limit psychotic behaviors due to abstinence.
Abstract ID 376
Determination of PTCA concentrations in hair by UHPLC-MS/MS and fluorescence techniques: a study on a wide multi-ethnic population with forensic application
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Aim
Nowadays forensic hair analysis plays an important role in assessing abstinence from drugs or ethanol abuse. Over the years, several studies have shown that many factors are likely to affect the content of xenobiotics in hair and thus complicate the interpretation of analytical results1. These factors include melanin content, physicochemical and structural properties of drugs, external contamination, but also cosmetic hair treatment. Indeed, one of the major drawbacks in hair analysis is the affectability of deposited xenobiotics by cosmetic treatments which could be eventually used to adulterate the sample. It is well known2 that some cosmetic treatments containing hydrogen peroxide (H₂O₂), such as permanent coloring or bleaching, lead to the formation of 1-H-pyrrole-2,3,5-tricarboxylic acid (PTCA), a melanin degradation metabolite. Considering that PTCA is an endogenous compound, spontaneously formed by natural oxidation of melanin, its only detection in hair is not enough to confirm a cosmetic treatment. For this reason, a study on PTCA endogenous level in a wide multi-ethnic population was performed.

Methods
In the present study, an ultra-high-performance liquid chromatographic–tandem mass spectrometry (UHPLC–MS/MS) procedure for the assay of PTCA was developed and validated in order to study its level in different hair samples and treatment conditions. First, PTCA was determined in natural (not treated) hair samples (n = 166) belonging to different ethnic groups: Caucasians, Indians, Blacks, Half-castes, Arabians and Asians. After that, a typical cosmetic treatment (Wella, Darmstadt, Germany) containing 12% H₂O₂ was applied to different natural hair samples for 30 minutes, according to official recommendations. Finally, since it is demonstrated that melanin oxidation leads to an increase in fluorescence emission3, natural and treated hair samples from the previously mentioned 6 ethnic groups were inspected by fluorescence microscopy.

Results
PTCA baseline level were detected as follows: Caucasians (2.26 ± 1.01 ng/mg, mean ± SD, n = 26); Indians (6.70 ± 3.00 ng/mg, n = 25); Blacks (6.85 ± 3.39 ng/mg, n = 24); Half-castes (3.87 ± 1.63 ng/mg, n = 29); Arabians (4.79 ± 2.81 ng/mg, n = 31); Asians (4.69 ± 2.61 ng/mg, n = 31). After 30 minutes incubation of hair samples in the dark with hair bleaching products containing 12% H₂O₂, the PTCA content increased by 500%. Cosmetic treatment did not provide any changing in PTCA content in light-blond as well as white hair.

Discussion
Lower PTCA baseline level was found in Caucasians, while higher one in Indians and Blacks. Moreover, the levels detected in Caucasians were found to be significantly different compared to each other considered ethnic groups. All treated hair samples have shown a comparable percentage increase in PTCA concentration, except for naturally low melanin content sample such as light-blond or white hair. The increase of the PTCA content in comparison to the untreated hair was statistically significant (p < 0.001). The changing in PTCA concentration, previously determined by UHPLC-MS/MS, were found to be directly correlated with the observed autofluorescence intensity.

Conclusions
In order to obtain a clear evidence of an applied cosmetic hair treatment, we aim to analyze in the next future a greater number of non-treated hair samples to establish a PTCA cut-off level as diagnostic marker in forensic field.

References
Abstract ID 386
A simple method for the determination of lacosamide in blood by GC-MS.

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Aim
Lacosamide ((R)-2-acetamido-N-benzyl-3-methoxypropionamide, LCM) belonging to the third generation AEDs, is a functionalized amino acid. It has 100% bioavailability after oral administration, peak concentrations in plasma are reached 0.5 to 4 hours after oral administration while it has a half life of 13 hours. Therapeutic plasma concentrations range from 2.0 to 15.0 µg/mL, while no toxic or fatal concentrations have been reported in the literature. It is important for a clinical/toxicology laboratory to analyze biological samples to determine lacosamide concentrations for TDM and for intoxication cases using a reliable, simple and rapid method. The aim of the study was to develop and validate a quick and efficient sample preparation method using liquid-liquid extraction following by GC-MS analysis for the determination of lacosamide in blood samples, without derivatization. Methods that have been reported for the determination of lacosamide in blood are mainly include LC-MS/MS instrumentation. GC-MS has been used for quantitation in blood in only case where a laborious sample treatment was applied by SPE and derivatization. The present method provides the advantage of a quick and reliable option appropriate for facilities that do not have access to LC-MS instrumentation.

Methods
Sample preparation was based on small blood sample volumes and minimal volumes of the extraction solvent with the intention to be used in routine toxicological analysis. Blood samples were used from lab’s routine samples, three clinical cases and one overdose fatal case (postmortem blood). Extraction of lacosamide was performed by a liquid-liquid extraction protocol with ethyl acetate at pH 12 which was selected based on the results of the experiments of the optimization for the extraction procedure. The analysis was performed within a 7 min run by an Agilent Technologies 7890A GC, combined with a 5975C MSD. Selected Ion Monitoring (SIM) detection was applied with the 91 m/z ion selected for quantitation (Q ion) of lacosamide and two additional ions 106 m/z and 163 m/z used for confirmation (C ions). As IS moclobemide was used by monitoring the ion 100 m/z ion.

Results
The method was found to be selective. The calibration curve was $y = (0.007±0.001)x-(0.01±0.003)$ with $R^2>0.998$. The values of LOD and LOQ were 0.1 and 0.5 µg/mL respectively. At LOQ, accuracy (R%) and precision expressed as RSD% were found to be 109.31% and 4.10% respectively. Intra assay accuracy ranged from 91.2% to 114.20% and precision from 4.62% to 9.27%, while inter assay accuracy ranged from 104.00% to 110.84% and precision from 8.01% to 10.14%. Short-term stability of the analyte in the samples kept at 4°C expressed as percentage recovery (%R) was found to be between 83.12% and 114.20% 24 hours after, between 78.56% and 121.90% 3 days after and between 81.31% and 119.42% 7 days after storage. Freeze thaw stability in the samples kept at -20°C was also within the acceptable limits (83.30-118.30%) for all concentration levels. The developed method was successfully applied in three routine clinical cases and in a fatal due to lacosamide overdose case. In the two clinical cases, lacosamide blood levels were found 8 and 9.5 µg/mL respectively (within the therapeutic levels, 2.0-15.0 µg/mL) and in the third, lacosamide blood concentrations were measured in three different times after intravenous administration and ranged between 0.6 and 4.7µg/mL. In the fatal case, lacosamide was determined in post-mortem blood at a level of 71 µg/mL.

Discussion
It is important for a clinical/toxicology laboratory to analyze biological samples to determine lacosamide concentrations for TDM and for intoxication cases using a reliable, simple and rapid method. As reported in literature, the clinical usage of the drug may be higher in the future thus the need for a reliable, sensitive and fast analysis method for the lacosamide is of particular importance. The suggested method will be further evaluated by participation to EQA program in order to be applied for routine analysis purposes.

Conclusions
The developed method was applied successfully in clinical and a post-mortem blood samples, indicating that can be a useful tool in TDM routine analysis. This research is one of few studies which determine lacosamide using gas chromatography-mass spectrometry without derivatization step, providing an immediate quantification of lacosamide in blood samples.
Abstract ID  388
Drugs of abuse in urine from patients with psychotic disorder.

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Aim
Drug abuse could represent a diagnostic challenge in patients presenting with psychotic symptoms, and could contribute to, or elicit, psychotic symptoms. Previous studies of patients admitted for psychiatric evaluation indicate frequent drug abuse in this population. However, the populations examined have often been heterogeneous, including patients with diverse psychiatric conditions. We wanted to evaluate drug use in a population of patients in which psychosis was the main diagnosis.

Methods
Urine samples were collected from 66 patients with newly diagnosed psychosis, included in the Bergen Psychosis Project 2 (BP2) between 2012 and 2017. The patients were supposed not to have used antipsychotic drugs prior to inclusion. Hydrolyzed urine samples were analyzed with LC-QTOF-MS using a data-independent screening method (SWATH). The samples were analyzed with a panel of 180 analytes, including traditional drugs of abuse and 53 new psychoactive substances (NPS).

Results
27 out of 66 urine samples (41%) were positive for drugs of abuse. 20 samples were positive for benzodiazepines and/or z-hypnotics, with oxazepam (24%), diazepam (12%) and zopiclone (9%) most frequently detected. 14 samples (21%) were positive for illegal drugs, with tetrahydrocannabinolic acid most frequently detected (15%), and followed by amphetamines (8%). No NPS were detected.

Discussion
As in previous studies, benzodiazepines, tetrahydrocannabinolic acid and amphetamines were the most frequently detected drugs in patients admitted to psychiatric ward. We cannot rule out that benzodiazepines and z-hypnotics were prescribed prior to urine sampling. Though the number of study subjects is limited, it seems like the prevalence of tetrahydrocannabinolic acid and amphetamines is somewhat lower than in previous studies. One explanation could be that the patient population in the present study is more homogeneous, with results reflecting the prevalence of drug abuse in a more exclusive group of patients suffering from psychosis. Improved capacity in drug addiction treatment in Norway during recent years could also be a contributing factor, with patients with drug addiction as their main problem being treated in institutions specializing in addiction medicine rather than in psychiatric institutions. Because of a continuously shifting NPS market, it is challenging to have an up-to-date analytical repertoire. Although no NPS were detected, it is possible that study subjects used NPS that were not included in the analytical method.

Conclusions
The present results show that benzodiazepines, tetrahydrocannabinolic acid and amphetamines are the most frequently detected drugs in a population of patients with psychosis. NPS were not detected in any of the samples, indicating a limited use of these substances in this population.
Abstract ID 426
A GC-MS method for the determination of five NPS in whole blood and their detection in urine.

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Aim
New psychoactive substances are gaining more and more space in illicit drug markets worldwide. Many NPS are not included in the list of substances controlled under Greek criminal law. NPS are classified into different chemical classes, due to their heterogeneous chemical structures. They are often designed to have similar structures with their “traditional” and banned analogues, so as to display similar pharmacological actions and to be ‘legal’. The aim of this project was to develop and validate a method for the detection and quantification of five NPS belonging to three categories: synthetic cathinones (mephedrone, 3,4-MDPV), opioids (AH-7921) and cannabinoids (JWH-018, AM-2201).

Mephedrone, 3,4-MDPV, AH-7921, JWH-018 and AM-2201 were quantified in whole blood samples and the same compounds plus methylone were detected in urine samples by GC-MS.

Methods
All calibrators and QCs were prepared by fortification of pooled blank blood and urine samples with methanolic mixtures of the compounds. The analytes were extracted by Liquid-Liquid Extraction from the biological matrices. 500 μL of the biological sample were used for the extraction, followed by evaporation of the organic phase and reconstitution with 50 μL of ethyl acetate. Selected Ion Monitoring was applied for the quantification of the analytes in a GC-MS system using an Agilent J&W HP-5ms Ultra Inert chromatographic column.

Results
The study for the optimum sample preparation method showed that derivatization was not necessary for the detection and quantification of the compounds as they could be detected with adequate sensitivity in the samples. The method showed good linearity for all analytes within a concentration range from 0.25 to 2 μg/mL for mephedrone and methylone, from 0.02 to 0.16 μg/mL for 3,4-MDPV and AH-7921 and from 0.005 to 0.04 μg/mL for JWH-018 and AM-2201. LOD for mephedrone was 0.23 μg/mL in blood and for mephedrone and methylone was 0.08 μg/mL in urine, for 3,4-MDPV was 0.017 μg/mL in blood and 0.008 μg/mL in urine, for AH-7921 was 0.016 μg/mL in blood and 0.01 μg/mL in urine, for JWH-018 was 0.004 μg/mL in blood and 0.003 μg/mL in urine, for AM-2201 was 0.004 μg/mL in blood and in urine. LOQ was 0.25 μg/mL for mephedrone, 0.02 μg/mL for 3,4-MDPV and AH-7921, 0.005 μg/mL for JWH-018 and AM-2201 in blood. Accuracy was within acceptable limits with %bias ranging from +20% to -19.91% for intra-assay study and from +18.87% to -18.89% for inter-assay study. Precision was found to be between 2% and 17% (CV%) for intra-assay study and from 2% to 14% (CV%) for inter-assay study. The interlaboratory assessment was performed between the Laboratory of Forensic Medicine & Toxicology (Department of Medicine, Aristotle University of Thessaloniki) and the Laboratory of Toxicology (Forensic Service of Ministry of Justice of Thessaloniki) for three samples containing all compounds at three concentration levels. The results of the two laboratories agreed with a correlation coefficient of 0.9954. No matrix effect or carryover was observed in both matrices, whereas the analytes were found to be stable in samples during two freeze-thaw cycles at -18 oC and in the sample extracts at 4 oC for two days. Specificity was evaluated in blank blood and urine samples after spiking a mix of MDMA, MBDB, methadone, cocaine, cannabidiol, Δ9-THC and heroin at concentration of 10 μg/mL.

Discussion
During sample preparation no derivatization step was performed, because even if mephedrone and methylone give derivatives with HFBA, the other compounds do not give derivatives and are negatively affected under the conditions of the derivatization. The developed method enables qualitative and quantitative determination of five NPS that belong to different chemical classes in blood and urine. Moreover, methylone can be detected in urine, but the detection of methylone in blood is impeded due to coelution of an endogenous substance.

Conclusions
The developed method can be used for the reliable and fast quantification of five NPS in blood and the detection of six NPS in urine; thus, it could be a supporting tool in a clinical or forensic toxicology lab.
Abstract ID 449
Determination of perampanel in human serum by capillary electrophoresis with fluorescence detection.

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Aim
Perampanel represents a new class of antiepileptic drugs for the adjunctive treatment of paediatric patients (age ≥ 12 years) with partial seizures and generalized tonic–clonic seizures associated with epilepsy. Given a significant linear relationship between efficacy (seizure reduction) and increasing perampanel plasma concentrations individualisation of perampanel dosing to clinical effect guided by therapeutic drug monitoring (TDM), is a vital strategy. Although liquid chromatography is well established in clinical laboratories, the use of capillary electrophoresis (CE), in particular in TDM settings, is on rise.

Methods
A sensitive CE method with on-line sample preconcentration by large volume sample stacking has been developed for determination of perampanel with fluorescence detection. CE separations were performed in a fused-silica capillary (Composite Metal Services, UK), inner diameter 50 µm, total/effective length 35/17 cm, which inner surface was covered using INST coating solution to reduce electroosmotic flow. The optimized background electrolyte consists from 50 mM chloroacetic acid with addition of 0.5% m/v polyvinylalcohol (pH 2.15); the separation is driven by maximal voltage +30 kV. Model or plasma samples treated by addition of 75% v/v acetonitrile were injected into the capillary in large amount that correspond to the length of sample zone equaled 129 mm (hydrodynamic pressure impulse 6000 mbar.s). Fluorescence detector (ARGOS 250 B, Flux Instrument, Switzerland) with a broad excitation filter 240 - 400 nm and a emission filter 495 nm was used for visualization of a native fluorescence perampanel.

Results
The developed large volume sample stacking technique is based on the forcing the sample zone out of the capillary with simultaneous application of separation voltage. Under such conditions, the enhancing factor achieves the value 57 for peak area compared to low injection. This approach allows the determination of perampanel in human blood plasma using only 25 µL of plasma treated by the addition of acetonitrile in a ratio of 1:3 v/v. The calibration dependence of the method was proved to be linear in the range of 10 - 1000 ng mL-1, with adequate accuracy (93.9 – 108.2 %) and precision (11.9 %). LOD and LOQ equaled 3 ng mL-1 and 10 ng mL-1 for perampanel, respectively.

Discussion
Our method offers a promising alternative for determining serum perampanel with several advantages. In particular, the low quantity of serum (25 µL) required means that testing can be performed on samples obtained for monitoring other antiepileptic medications, and thus reduces the test-burden on paediatric patients. The newly developed methods performance was tested on a series of serum samples from patients treated with perampanel and the results corelated with reference LC-MS/MS method.

Conclusions
The present CE-FD method for the determination of perampanel in human serum is advantageous over existing methods in terms of simple sample precipitation, high-throughput sample processing short retention times, high specificity and excellent sensitivity provided by fluorescence detection. The new type of large volume sample stacking achieves detection limits comparable with those of LC-MS/MS while using minimal serum sample volumes.

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Abstract ID 451
Mercury, what to expect when it is being analyzed?
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Aim
In 2013 Uruguay ratified the Minamata Agreement with other 107 countries and one year later it was approved by law. This agreement implies the elimination of Mercury in the environment, therefore, all the technology based on the use of this metal must be eliminated in an appropriate way and replaced by other environmentally friendly, which is the basis of the GEF / UNDP Project "Environmental Management Adequate Products and Residues Containing Mercury" with other Uruguayan organizations as Ministerio de Vivienda, Ordenamiento Territorial y Medio Ambiente, Dirección Nacional de Medio Ambiente, Ministerio de Salud Pública, Programa de las Naciones Unidas para el Desarrollo y la Agencia Uruguaya de Cooperación Internacional.

This project as well promotes the population study "Average level of Mercury in pregnant women and newborns in Uruguay" since 2017, on which the Unit participates with the Centro de Información y Asesoramiento Toxicologico, Facultad de Medicina, and Ministerio de Salud Pública. It should be noted that in Uruguay no study on the levels of this metal in its population has been carried out, so the lack of information is one of the reasons that motivate this study.

Methods
In addition, with the other participating members, it was chosen to focus the study on the most vulnerable population, pregnant women in the last trimester and newborns, all of the patients of Public Institutions. From this participants were taken samples of blood, hair, and urine in case of mothers and umbilical cord blood in newborns, previously agreed on an informed consent, which was approved by the ethics committee of the Hospital de Clínicas.

All analyzes were performed at the Unit's facilities using a Direct Mercury Analyzer, DMA-80, Milestone against certified reference materials of the International Atomic Energy Agency and the National Institute Standards and Technology. To the date, a total of 933 samples were collected and analyzed (66% of the total planned), 220 correspond to urine, 218 to hair, 357 to blood and 138 to cord blood.

Results
From the values obtained, the mean values of blood, hair, urine and cord blood the values obtained are lower than those reported by some countries or regions that have already carried out this study and those stipulated by international organizations such as the World Health Organization. Although we found cases of values higher than those of international reference, they are less than 1% of the samples analyzed.

Discussion
We must not lose sight of the fact that the eating habits of Uruguay are what define in some way this mercury levels. In addition, the fact that this study is about pregnant women and newborns in vulnerable situations implies having focused the study on a very limited sample, and therefore the values obtained can not be extrapolated to any other kind of population. Therefore, this is simply the starting point for future studies on mercury levels in the Uruguayan population.

Conclusions
This study is extremely relevant to establish a baseline of information on mercury levels in the Uruguayan population, generating useful information for public health and knowledge for future studies.
Abstract ID 465
LC-MS/MS determination of 20 anti-psychotic drugs and metabolites.
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Aim
According to statistics from the National Police Agency in South Korea over the past five years, the recidivism rate for mentally-disordered criminals is about 20 percent higher than the total recidivism rate, especially for violent crimes such as murder and rape, which are up to 9 percent higher. As a countermeasure to prevent recurrence of mentally-disordered criminals, a medication compliance monitoring guideline has been introduced, and implemented in criminal justice systems in South Korea since January 2019. The Court legally orders a mentally-disordered criminal to receive medication treatment, and the Probation Office is responsible for monitoring medication compliance of a subject. A simultaneous analytical method for 20 anti-psychotic drugs and metabolites in urine using LC-MS/MS has been developed for complying medication compliance monitoring system.

Methods
20 anti-psychotic drugs of the first, second and third generations as well as anti-psychotic drug with more than 10,000 prescription cases in South Korea were selected. Sample preparation was achieved by using a solid-phase extraction(Oasis HLB, 60 mg) method to remove interference and minimize matrix effect in 1 mL urine samples. An LC-MS/MS analysis was performed in the positive ESI mode in an AB Sciex API 4000 triple quadrupole MS/MS system and Agilent 1290 UPLC system. UPLC separation was performed using a Waters Xselect HSS T3(2.1×150 mm, 2.5 μm) column and a gradient elution was achieved using 0.1% formic acid in water and 0.1% formic acid in methanol.

Results
All 20 target drugs were separated and analyzed within 15 minute and the method was validated according to U.S. FDA Bioanalytical Method Validation. Selectivity, linearity(R2≥0.9938), limit of quantification(0.1~2.0 ng/mL), accuracy(-9.4~10.4%), precision(CV≤14.0%), recovery(60~114%), matrix effect, integrity of the dilution, and stability of samples in laboratory conditions were evaluated and all validation parameters were met verification criteria.

Discussion
We analyzed urine sample for 39 subjects of medication compliance monitoring system, and the second generation anti-psychotic drugs such as Quetiapine, Risperidone, Olanzapine and the third generation drug Aripiprazole were most detected. Concentration of Quetiapine was 0.6 to 160 ng/mL, Risperidone was 16 to 465 ng/mL, Aripiprazole was 0.8 to 34 ng/mL and Olanzapine was 1.9 to 230 ng/mL in real sample. In urine sample of 3 subjects, no anti-psychotic drugs have been detected, which can be interpreted as not complying with medication compliance monitoring system.

Conclusions
In this study, an analytical method based on LC-MS/MS was developed and validated for 20 anti-psychotic drugs and metabolites in urine. We analyzed anti-psychotic drugs in urine sample of mentally-disordered criminals by using this method, which allowed verifying that the subject is properly complying with medication treatment order of the Court. Thus, developed method is an effective way to increase effectiveness for medication compliance monitoring system.
Abstract ID 469
Evaluation on the performance of Lin-zhi 6-Acetylmorphine Enzyme Immunoassay (EIA) on Roche Cobas c501 Chemistry Analyser.
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Aim
In the laboratory, the screening for common drugs of abuse such as opiates, amphetamines, cannabinoids, benzodiazepines, cocaine, methadone, phencyclidine, propoxyphene, barbiturates and methaqualone in urine were performed using immunoassays based on kinetic interaction of microparticles in solution (KIMS) on Cobas c501 chemistry analyser (Roche Diagnostics). However, the screening of 6-acetylmorphine (6-AM) in urine utilises a separate assay, Microgenics CEDIA Heroin Metabolite (6-AM) immunoassay on an Olympus AU800 chemistry analyser (Beckman Coulter). To streamline the drugs screening workflow, the laboratory explored to use the 6-AM Enzyme Immunoassay (Lin-Zhi International, Inc.) on Cobas c 501 chemistry analyser to consolidate all common drugs of abuse screening onto a single chemistry analyser.

Methods
The Lin-Zhi 6-AM Enzyme Immunoassay (EIA) was evaluated for the detection of 6-AM on a Cobas c 501 chemistry analyser. The analyser was calibrated using the 5 calibrators at 0-40 ng/ml and assessed by the reagent controls containing 7.5 (negative control) and 12.5 ng/ml of 6-AM (positive control) daily prior to sample analysis. The assay was validated in accordance with the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines for immunoassay-based screening methods. Parameters assessed include precision, selectivity, cross-reactivity and dilution integrity. A total of 61 authentic urine samples from opiate abusers that were previously submitted to our laboratory were evaluated using the CEDIA 6-AM assay and Lin-Zhi 6-AM Enzyme Immunoassay at 10 ng/ml cut-off for comparison. These samples contained 6-AM concentrations ranging from 6.0 to 674.1 ng/ml and morphine concentrations ranging from 584 to 10,670 ng/ml, as quantified by LC-MS/MS and GC-MS, respectively.

Results
The within-day and between-day precisions for 6-AM at concentrations of ± 50% from the cut-off at 10 ng/ml were found to be <7% and <6%, respectively. The on-board dilution integrity evaluated for dilution factors of 5, 10 and 20 were found to be < 9%. The calibration of Lin-Zhi 6-AM EIA was found to be stable for up to 30 days. This is an advantage over the CEDIA 6-AM assay where the calibrator was determined to be stable for up to about a week. From the parallel study of 61 samples, Lin-Zhi 6-AM EIA demonstrated comparable sensitivity to CEDIA 6-AM assay, and with better specificity for 6-AM (100.0% vs ~96.7% on CEDIA)). Of the 61 sample, CEDIA 6-AM assay generated two false positive results for samples containing 6.24 and 6.04 ng/ml 6-AM, respectively. Upon investigation, the CEDIA 6-AM assay can cross-react with morphine when found present in the urine sample. No cross-reactivity against morphine was observed for Lin-Zhi 6-AM Enzyme Immunoassay up to 100 µg/ml. The cross-reactivity of the Lin-Zhi 6-AM assay was also evaluated against other common opiates encountered in Singapore and found to have no cross-reactivity with codeine up to 800 µg/ml, dihydrocodeine up to 750 µg/ml and dextromethorphan up to 200 µg/ml, which performed according to what is stated in Lin-Zhi’s 6-AM assay product insert.

Discussion

Conclusions
The performance of Lin-Zhi 6-AM EIA on Cobas c501 was found to be sensitive and specific for the detection of 6-AM without significant cross-reactivity with morphine. The implementation of this assay on Cobas c501 had improved the efficiency of drugs screening workflow in the laboratory.
Abstract ID 472

Alternative Forensic Matrices: Evaluation of Simplified Workflow for Drugs of Abuse Extraction from Nail Samples Prior to LC-MS/MS Analysis.

Katie-Jo Teehan
Katie-Jo Teehan, Lee Williams, Rhys Jones, Adam Senior, Helen Lodder, Geoff Davies, Alan Edgington, Steve Jordan, Claire Desbrow, Paul Roberts
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Aim
The testing of alternative matrices in forensic and/or clinical toxicology is gaining popularity, partly due to less invasive means of collection. Blood and urine testing are still by far more prevalent. However, traditional testing in combination with other matrices such as hair or nail can provide a more rounded picture of abstinence or abuse and associated timeframes. Solid matrix analysis by LC/MS or GC/MS is generally more involved due to the necessity of multiple manual labour steps to convert the sample into an extractable form. This poster aims to demonstrate workflow advantages for fingernail analysis from multi-sample matrix homogenization, extraction and analysis for a range of drugs of abuse classes.

Methods
Nail samples (10 mg) were subjected to dry micro-pulverization using the Biotage® Lysera bead mill homogenizer. The nails were ground to a fine powder using a protocol of 8 cycles at 6.95 m/sec for 45 seconds with a dwell time of 45 seconds. Samples were then soaked in 1 mL of methanol prior to clean-up using ISOLUTE® SLE+ 400 µL capacity supported liquid extraction columns. Manual positive processing was compared to the Biotage® Extrahera™ automated sample preparation platform. LC-MS/MS analysis was performed using a Shimadzu Nexera UHPLC system coupled to an 8060 triple quadrupole mass spectrometer. MRM transitions were selected using the most intense precursor ions. LC mobile and stationary phases were selected based on analyte retention, resolution, symmetry and MS signal to noise. Final chromatography was performed using a Restek Raptor Biphenyl HPLC column with a combination of 2 mM ammonium formate and 0.1% formic acid as additives in both the aqueous and organic mobile phases.

Results
A typical panel of drugs of abuse including amphetamine and related analogues, ketamines, benzodiazepines, Z-drugs, cocaine, opiates, fentanyl and buprenorphines was investigated.

Initial nail extraction comparing dry and direct solvent-based homogenization was investigated. Due to the hard nature of the matrix better sample pulverization was achieved using dry samples. Nail extracts were then soaked in solvent and mixed further for effective drug transfer into solution. Methanolic solutions were selected for optimum solubility and drug transfer along with pH modification to aid subsequent extraction methodologies. Sample clean-up was performed using ISOLUTE SLE+ supported liquid extraction columns. Comparison of streamlined methodology loading the neat nail homogenate versus pre-concentration prior to loading was fully investigated. SLE optimization investigated loading pH and extraction solvent combinations from a recovery and suppression standpoint.

Discussion
Screening results indicated that in order to get the best extraction of multi-class drugs of abuse ISOLUTE SLE+ extraction required an aliquot of 95/5 DCM/IPA (v/v) followed by a subsequent aliquot of MTBE. The majority of analytes delivered extraction recoveries > 80% with all analytes demonstrating RSDs < 10%.

Calibration curves were constructed using fingernail samples spiked between 1-1000 pg/mg. Results demonstrated excellent linearity and coefficients of determination (r²) greater than 0.99 for all analytes. LoQs were typically < 1 pg/mg for the majority of analytes in the panel. Sub 10 pg/mg levels were achieved for all other target analytes. Full comparison of the direct and pre-concentration extraction methodologies in terms of recoveries, RSDs, signal response, calibration curves and LOQs showed similar performance for most analytes. BZE recovery was substantially different between the methods. Extrahera processing allowed an automated extraction procedure directly comparable to offline positive pressure processing. However, in all cases the desired levels were met using both methodologies. Full details of the optimized workflow will be demonstrated.

An increase in temperature could degrade the amphetamines, however the Biotage® Lysera bead mill homogenizer can be used with a Cryo unit which will prevent such a high increase in temperature.

Conclusions
This poster demonstrates simplified workflows for the extraction and analysis of multi-class drugs of abuse from fingernail samples.
Abstract ID 475
Screening and searching the poisoning biomarkers of Aconitum alkaloids in rat blood and urine based on metabolomics.
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Aim
Aconitum species were used as useful herbal medicines in traditional Chinese medicine (TCM). Aconitum alkaloids from Aconitum species plants not only have a wide range of pharmacological effects, such as analgesic, diuretic, anti-inflammatory and cardiotoxic actions but also have strong toxicity. Because of processed aconite roots with a narrow therapeutic window, improper preparation or overdose could lead to acute poisoning or even death. The absorption and metabolism of aconitum alkaloids were quick, original medicine were not been detected in this kind of poisoning cases. Therefore, many cases of accidental and intentional intoxication from Aconitum species had been reported sometimes. In order to distinguishing between taking poison before death and postmortem poisoning, it is necessary to search potential poisoning markers based on metabolomics. Non-targeted metabolomics was mainly used to compare the metabolomics of the control groups and the treated groups, in order to find the differences metabolites and explain their biological significances.

Methods
Aconitine (PC-01), mesaconitine (PC-02), hypaconitine (PC-03) and the control groups (WT-01) in this study to train rats poisoning model by gavage, respectively. Blood and urine of the control groups and the treated groups were collected, the potential biological differences were detected by LC-MS/MS. Multivariate statistical analysis, such as principal component analysis (PCA) and partial least squares discrimination analysis (PLS-DA) were used to screen differentiated metabolites, and the boundary value was set as p-value (0.05)and VIP (1). Subsequent to the Human Metabolome according to MS/MS fragmentation mode Database (HMDB), Metlin, massbank and other databases were used to confirm and annotate metabolites. Metabolic pathways were analyzed with KEGG database, and metabolic network model was visualized by metPA network software. When metPA analysis is carried out, impact value (0.1) was set as the boundary value from the literature, so the access method has research significance.

Results
In the urine matrix, four significant metabolites and six kinds of potential metabolic pathways were found, mainly involving the synthesis and metabolism of amino acids, by comparing to the results of aconitine, mesaconitine, hypaconitine and the control group. In the blood matrix, eight significant metabolites and seven kinds of metabolic pathways were found, mainly involving aerobic respiration, amino acid synthesis and metabolism.

Discussion
The potential metabolic differences and metabolic pathway were screened out by non-target metabolomics in this study. The results showed that the same metabolic pathways was found in the multiple comparison group. The biosynthetic pathways of phenylalanine, tyrosine and tryptophan were observed in the four groups of urine, such as PC-01 vs. PC-02, PC-01 vs. PC-03, WT-01 vs. PC-01, WT-01 vs. PC-01 vs. PC-02 vs. PC-03. In the PC-01 vs PC-03 control group of urine, the differentiated metabolite of tyrosine simultaneously appeared in the biosynthetic pathway and tyrosine metabolism of phenylalanine, tyrosine and tryptophan.

Conclusions
In order to further study on three compounds metabolic pathway (aconitine, mesaconitine, hypaconitine) in SD rats, target metabolomics approaches will be applied in follow-up study.

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Abstract ID 477
Identification of substances in emergency room patients.
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Aim
The number of poisoned patients ranged 0.66 ~ 1.3 % of total number of patients in the emergency room in Korea. Because there has been no study conducted to identify toxicants in biological fluids, we examined the biological fluids from poisoned patient to determine the toxicants in patients.

Methods
A total of 120 patient samples of urine and blood were collected from emergency room. To identify toxicants, samples were extracted by liquid-liquid extraction (LLE) and examined by GC-MS and LC-QTOF-MS. The GC-MS system was composed of an Agilent 7890B GC combined with an Agilent 5977 A inert MSD. The LC-QTOF used for the analysis consists of the Agilent 1290 series LC and the Agilent 6545 QTOF. After analysis by GC-MS, a library search was conducted by an in-house library established with the automated mass spectral deconvolution and identification system (AMDIS). LC-QTOF-MS was used for screening with a custom library using MassHunter Personal Compound Database and Library (PCDL) Manager.

Results
91 different drugs and 15 pesticides were identified in 120 samples. Among drugs, Zolpidem, acetaminophen, tramadol, doxylamine and alprazolam were the most frequently encountered with 28, 23, 17, 10 and 9 frequencies. For pesticides, diazinon and glufosinate were detected in five cases while others were detected only in one case. For drug intoxicants, anti-inflammatory drugs including acetaminophen were the most common intoxicants, accounting for 23.3%, and sleep depressants including zolpidem were 16.2%. For pesticide intoxicants, 53.8% insecticides, 30.8% herbicides and 11.5% disinfectants were detected.

Discussion
Polydrug use was noted in many cases. 2 items were detected in 24 cases, 3 items in 17 cases and 5 items in 7 cases. There were 23 cases in which no drug and pesticides were detected while in 31 cases only one drug was determined.

Conclusions
Through the developed analytical method, it was possible to effectively identify drugs in biological samples of emergency room patients. This method could be useful in clinical toxicology as well as in forensic toxicology practices.

Abstract ID 493
The metabolism of the fentanyl analogues 3-methylcrotonylfentanyl, furanylbenzylfentanyl and 4-fluorobenzylfentanyl.
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Aim
The opioid analgesic fentanyl and its analogues pose a major health concern due to their high potency and increasing number of overdose deaths worldwide. As new fentanyl analogues are continuously emerging much is still unknown considering the metabolism of these compounds. In clinical and forensic toxicology, metabolite profiling can be an important tool for analytical identification. 3-methylcrotonylfentanyl, furanyl-benzylfentanyl and 4-fluorobenzylfentanyl are three fentanyl analogues that were seized at European borders in 2017 and 2018. The aim of this study was to identify the main metabolites of these compounds.

Methods
Human liver microsomes and cryopreserved human hepatocytes were incubated with 3-methylcrotonylfentanyl, furanylbenzylfentanyl or 4-fluorobenzylfentanyl at a final concentration of 1 µM for 0, 0.5, 1, 2 and 4 hours before the metabolic reactions were stopped by adding formic acid. The formed metabolites were separated by ultra-high performance liquid chromatography using an Acquity HSS T3 column (2.1 x 100 mm, 1.8 µm, Waters) employing gradient elution with a mobile phase consisting of 10 mM ammonium formate pH 3.1 and MeOH. Identification of the compounds was performed by quadrupole time-of-flight mass spectrometry analysis (6550 iFunnel Q-TOF LC/MS, Agilent).

Results
The main metabolites of 4-fluorobenzylfentanyl were generated by N-dealkylation and hydroxylation. Furanylbenzylfentanyl metabolism was dominated by amide hydrolysis or dihydrodiol formation when incubated with hepatocytes, and amide hydrolysis followed by hydroxylation or dihydrodiol formation when incubated with microsomes. The metabolites of 3-methylcrotonylfentanyl were mainly formed by N-dealkylation or hydroxylation upon incubation with hepatocytes, and hydroxylation or carboxylation after microsome incubation.

Discussion
The metabolism of 4-fluorobenzylfentanyl and 3-methylcrotonylfentanyl appears to be similar to that of fentanyl which is dominated by N-dealkylation. Furanylbenzylfentanyl metabolism is more like that of furanylfentanyl which is dominated by amide hydrolysis and dihydrodiol formation at the furanyl ring system. The major metabolites of the three analogues formed by human liver microsomes and hepatocytes differed slightly regarding which biotransformations occurred and the ratio between metabolites formed. However, the two in vitro models also formed shared metabolites for each compound which can be employed for accurate detection of the parent compounds.

Conclusions
In the present study we successfully identified the metabolites of 3-methylcrotonylfentanyl, furanylbenzylfentanyl and 4-fluorobenzylfentanyl which can aid in the identification of these compounds in forensic laboratories.
Abstract ID 494
Simplified Sample Preparation for Low Level Determination of Cannabis Use from Hair Samples Prior to LC-MS/MS Analysis.
Katie-Jo Teehan
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Biotage GB

Aim
Hair analysis is growing in popularity due to the non-invasive nature of the sample collection. Although not used routinely as per 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. Furthermore, the workflow will be demonstrated for application to the low levels required for cannabis testing from this matrix.

Methods
Hair samples (20 mg) were subjected to micro-pulverized extraction in methanol using the Biotage® Lysera bead mill homogenizer. Methanolic extracts were cleaned-up using supported liquid extraction: ISOLUTE® SLE+ in 400 µL capacity 96-well plates or equivalent columns. LC-MS/MS analysis was performed using a Shimadzu Nexera UHPLC system coupled to an 8060 triple quadrupole MS. MRM transitions were selected using the most intense precursor ions in positive or negative mode. LC mobile and stationary phases were selected based on analyte retention, resolution, symmetry and MS signal to noise. Final chromatography was performed using an ACE 2 C18 UHPLC column with a combination of 0.01% acetic acid as an additive in both the aqueous mobile phase and methanol as the organic eluent.

The following MRM transitions were selected for quantification; THC 315.0>193.1, THC-OH 331.0>313.3, THC-COOH 343.3>299.3, CBN 311.0>223.0, CBD 313.2>245.15 and THCAA 357.3>313.3.

Results
A panel of cannabinoids and metabolites were investigated, included: cannabinol (CBN), cannabidiol (CBD), Tetrahydrocannabinol (THC), 11-Nor-9-carboxy-Δ⁹-tetrahydrocannabinol (THC-COOH), 11-Hydroxy-Δ⁹-tetrahydrocarbinol (THC-OH), Δ9-tetrahydrocannabinolic acid-A (THCAA). Method development required investigation of LC mobile phases to achieve good chromatographic performance along with providing maximum positive and negative ion sensitivity. In this case a weakly acidic mobile phase utilizing 0.01% acetic acid in aq and organic solvent was selected. Previous work has identified the use of ammonium fluoride as an additive to maximize positive/negative ion sensitivity. However, in this case the acetic acid mobile phase yielded better performance.

Initial hair extraction was investigated to determine maximum matrix/solvent proportions. To achieve direct homogenization of the matrix in solution a 20 mg sample was selected with 1 mL of organic solvent. Methanol with or without pH modification was chosen for the extraction solvent due to hair swelling ability allowing effective analyte release from the matrix.

Discussion
Sample clean-up was performed using supported liquid extraction. A range of solvents were investigated from a recovery and suppression standpoint. Screening results indicated MTBE to be the best performing solvent. In order to reach SoHT LoQs we compared methanolic extract evaporation and reconstitution prior to extraction with direct extraction of the methanol extract. Typical recoveries were > 60% with corresponding RSDs < 10%. A range of extraction solvents were applicable depending on the exact panel required.

Calibration curves were constructed using hair spiked between 0.1-200 pg/mg. Results demonstrated good linearity and coefficients of determination (r2) values greater than 0.99 for all analytes. Similar performance was achieved using ISOLUTE SLE+ in the 400 µL capacity column as well as the high throughput 96-well plate formats. LoQs were below required SoHT guidelines for both screening and confirmation. THC-COOH achieved the 0.2 pg/mg levels set out with other cannabinoids reaching the between 1 and 10 pg/mL.

Conclusions
This poster demonstrates simplified workflows for the extraction and cleanup of hair matrix allowing low level determination for cannabis use.
Abstract ID  509
Importance of interpretation in forensic toxicology analysis results.

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Aim
Substance abuse is a problem that we are facing in many dimensions in the world. The presence of opiates in the urine sample of forensic toxicological cases can be caused by legal prescription drugs or by substance abuse. Codeine is one of the most commonly used opiates in the world with a wide safety margin. It’s found in combination preparations with acetaminophen, naproxen sodium etc. in many over-the-counter drugs used for cold symptoms and analgesia. Patients who took codeine have shown both codeine and morphine in their plasma and urine. Therefore, determining the origin of morphine found in urine samples of probationer or clinical cases can be challenging and interpretation of analysis results is gaining great importance. Furthermore, the relationship between substance abuse and the legal and judicial systems increases the responsibility of specialists in these toxicological stages. In this study, a probation case come from substance abuse (opiate) and had an opioid positive result was presented in order to demonstrate the importance of interpretation.

Methods
A 43 years old male probationer was admitted to Ege University BATI Institute, Addiction Toxicology Laboratory. According to Turkish Penalty Code, individuals who have received probation undergo a 6-week treatment program and are subjected to a urine drug screening test and a clinical interview. Probationer stated that he used pain killer (Apranax-Plus) regularly. He did not remember the number or the exact time of ingesting the tablets. Apranax-Plus is an nonsteroidal anti-inflammatory prescription drug and it contains 550 mg Naproxen sodium and 30 mg codeine. According to the routine methodology in the laboratory, six different urine samples were obtained from probationer under the chain of custody between April-June 2018. Urine samples were first screened for drugs of abuse using CEDIA (Cloned Enzyme Donor Immunoassay) reagents. Then, opiates results confirmed with Gas Chromatography-Mass Spectrometry (GC-MS). Morphine/codeine ratio was taken into consideration when interpreting the results to differentiate between consuming codeine and morphine.

Results
The analysis results were all found to be positive for opiates (cut off: 300 ng/ml) at the screening analysis. Heroin metabolite 6- acetyl morphone was not detected in all urine samples at the screening analysis. Sample integrity tests were normal for all urine samples. GC-MS results showed positivity for morphine, codeine and naproxen sodium. Mor/Cod ratio below 1 is considered as a sign of codeine only intake, whereas the ratio above 1 is considered as a sign of using morphine or heroin. But the number of one is not absolute to determine the source of morphine, some suggested that in individuals with ultra-rapid CYP2D6 metabolism, Mor/Cod ratio can be higher than 1 in even sole consumption of codeine. In this study, mor/cod area ratios were 1.45, 0.38, 0.88, 2.96, 3.57 and 0.99 for six urine samples, respectively. Naproxen-sodium has the highest peak height and area.

Discussion
The assessments of medication history plays a crucial role for the correct interpretation of the results. A study by He et al. in 2008 has shown that in the individuals with ultra-rapid CYP2D6 metabolism, Mor/Cod ratios were below 1, median (range): 0.108 (0.045 - 0.236) in plasma, 12 hours after codeine consumption. But it could be above 1, median (range): 0.635 (0.184 - 1.060) after 24 hours. In six urine samples of our case, mor/cod ratios were varied. Median of our case's results was 1.22 (range: 0.38 – 3.57). Detection of high levels of morphine in urine samples may be caused by codeine, because of it can convert into morphine.

Conclusions
Physicians should be able to determine whether the positive test results could be related to proper use of a prescription drug or from substance abuse. In our case, mor/cod ratio of the last urine sample was 0.99. The case can be considered as fast metabolizer but, further metabolic enzyme studies are required to make the final decision. The results demonstrated that there is a strong need for the careful interpretation of the analysis results by an experienced forensic toxicologist in the laboratories to reduce wrong results. A deeper understanding of toxicology testing can help clarify conflicting results.
Abstract ID 517
The development and validation of a method for serum Infliximab by liquid chromatography tandem mass spectrometry (LC-MS/MS).
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Aim
Infliximab (RemicadeTM) is an anti-TNF-α monoclonal antibody used in the treatment of a number of conditions including Crohn’s disease, ulcerative colitis and rheumatoid arthritis. For a number of reasons, including the presence of anti-infliximab antibodies, measurement of infliximab has been recommended to guide treatment. At present, all methods for infliximab measurement in the UK are via immunoassay which are expensive and may be prone to interferences typical of this technique. The aim of this project was to develop and validate a new method for the routine measurement of serum infliximab by liquid chromatography tandem mass spectrometry (LC-MS/MS). In this project two digestion methods were evaluated with Infliximab samples to produce signature peptides that could later be identified by LC-MS/MS. The two methods assessed in this project were a commercial kit and an in-house immunocapture procedure, both being compared to the in-house enzyme linked immunoassay (ELISA) currently used to measure Infliximab drug levels.

Methods
A novel LC-MS/MS method has been developed to quantify Infliximab drug levels using a signature peptide (SINSATH) specific to Infliximab. All analytical parameters were determined using both spiked Waters digestion buffer (ProteinWorks eXpress Digest Kit) and spiked serum samples with Infliximab which were analysed on a Waters TQS LC-MS/MS system using an I-Class Acquity UPLC. Serum samples were digested by both a surrogate peptide approach with a commercial kit and an in-house immunocapture method using biotinylated TNF-α. Specimen volumes were 25 µL and 2 µL respectively. The validated method was subsequently applied to the analysis of both previously analysed patient samples and external quality assurance (EQA) scheme samples to allow comparison to the current in-house ELISA method.

Results
The LC-MS/MS method was found to overcome interferences observed in the current in-house ELISA method alongside improved assay sensitivity and linearity. The assay was successfully validated and was found to be linear in a range of 0.1–100ug/L (R² = 0.988). The lower limit of quantification for the assay (<20% CV) was 0.1 µg/L requiring only a small volume of sample. There was clear agreement between the current in-house ELISA method and the newly developed LC-MS/MS method with regression analysis showing a correlation of R²=0.99. An overall recovery of 82% was observed and precision was within acceptable limits (<20%): within-batch precision 2.36% and between-batch precision 5.42%. In addition to this, stability studies were completed evidencing sample digest stability of 7-days at auto sampler temperature (2-8°C), a stability of 2-days at freezer temperature (-20°C) and instability at deep-freeze temperature (-80°C). In summary, the in-house immunocapture digestion method was found to be the most reproducible and financially beneficial to bring into service.

Discussion
The results outlined here show a new method for the measurement of infliximab in human serum that has been fully validated and can be brought into routine use. It offers a more efficient method that ELISA with a shorter, less complicated workflow along with improved assay sensitivity, specificity and clinical utility of results produced.

Conclusions
Therapeutic drug monitoring is important in the successful treatment of patients with Infliximab to prevent disease relapse and it is for this fundamental reason a clinically reliable assay has been produced. Through developing a new method this overcomes the interference seen in the current ELISA methods and the variability between hospital trusts.
Abstract ID 527

Discriminate “Brown Mixture” ingestion or heroin use by monitoring antimony in urine using inductively coupled plasma mass spectrometry.

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Chung Sum Medical University

Aim
Like to the “poppy-seed defense” commonly reported in the Europe and United States, urine testing positive of opiates in Taiwan often claimed the ingestion of Brown Mixture (BM) as the source of morphine and codeine. BM, a very popular expectorant with dark brown liquid made of fluid extract of licorice root, tartar emetic, camphorated tincture of opium, spirit of ethyl nitrite glycerol and water, used as a remedy in Asia. Because heroin consumption is considered a serious crime, discrimination between BM ingestion and street heroin consumption represents a major toxicological challenge. In this study, a novel solution was developed to differentiate BM and heroin use in urine by monitoring antimony using inductively coupled plasma mass spectrometry (ICP-MS).

Methods
In this study, the determination of morphine and codeine in urine were used gas chromatography mass spectrometry (GC-MS). The determination of antimony in urine was using (ICP-MS). The instrument was operated at an rf power setting of 1.30 kW. A standard demountable quartz torch with 1.2 mm id alumina injector tube was used with the following argon gas flow rates: nebulizer, 0.75 L/min, auxiliary, 0.85 L/min; and coolant, 15.0 L/min. Platinum sampler and skimmer cones were employed and these had orifice diameters of, respectively, 1.14 and 0.89 mm. The sample solution was introduced into a nebulizer via a peristaltic pump operated at a delivery rate of 3.0 mL/ min.

Results
BM contains antimony potassium tartrate as a vomitory, therefore a novel solution was developed to differentiate BM and heroin use by monitoring antimony in urine using ICP-MS. Urine specimens are prepared for analysis by simply diluting 200 μL sample volumes to 3 mL with water and adding indium as internal standard. All analyses were subjected to stringent internal quality control protocols. Detection limits were 0.01 ng/mL in urine. The recoveries in this study were 92.5-93.8%. Precisions of analyses were better than 5–10%. Urinary excretion of codeine, morphine and antimony following the adapted ingestion patterns of single and multiple doses of BM were showed. The excretion time curve of codeine, morphine and antimony was similar. In the single dose, the highest excretion period of morphine and codeine was occurred 2 hr and antimony at 4-6 hr following BM ingestion. Max concentration of antimony (range 22.5-29.7 ng/mL) was obviously different in BM ingestion and heroin use. More significantly, all samples that contained morphine at the concentration above 300 ng/mL were shown to also contain measurable amounts (several ng/mL), and the blank urine was below 0.1 ng/mL of antimony. Max concentration of antimony were between 22.5-29.7 ng/mL. There was obviously different in BM ingestion and heroin use.

Conclusions
A novel solution was developed to differentiate BM and heroin use in urine by monitoring antimony using ICP-MS with the detection limits 0.01 ng/of antimony in urine. All BM urine samples that contained morphine were shown to contain several ng/of antimony, but the blank urine were below 0.1 ng/mL. Those results show that it is sufficiently differentiated BM ingestion and heroin use by monitoring antimony in urine using ICP-MS.

Discussion
Because BM contains opium powder, opium tincture, or camphorated opium tincture, there is still a big challenge to distinguish whether morphine in urine, in the absence of 6-monoacetylmorphine (6-AM), originates from heroin use or BM ingestion. The used [M]/[C] ratio might not be an effective parameter to differentiate heroin use from BM ingestion. It appeared that BM ingestion was unlikely to result in a morphine concentration > 4000 ng/mL, but was found > 4000 ng/mL in many real cases. BM contains antimony potassium tartrate as a vomitory, therefore a novel solution was developed to differentiate BM and heroin use in urine by monitoring antimony using ICP-MS. Methods are presented for the determination of antimony in urine by ICP-MS. All samples that contained morphine at the concentration above 300 ng/mL were shown to also contain measurable amounts (several ng/mL), and the blank urine was below 0.1 ng/mL of antimony. Max concentration of antimony were between 22.5-29.7 ng/mL. There was obviously different in BM ingestion and heroin use.
Abstract

Cross reactivity of the CEDIA and HEIA benzodiazepine kits for 29 designer benzodiazepines and tofisopam.

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Aim
The number of designer benzodiazepines that have emerged on the world wide drug market has increased constantly over the last couple of years. In contrast to other classes of new psychoactive substances, such as cannabinoid receptor agonists, immunochemical assays have shown to be a suitable tool for the detection of older designer benzodiazepines in biological samples [1]. However, data on newly emerging compounds such as flualprazolam, phenazolam and fluclozolam is missing.

The aim of the present study was to assess the cross-reactivity of 29 designer benzodiazepines as well as of the 2,3-benzodiazepine tofisopam utilizing the CEDIA and the HEIA benzodiazepine assays.

Methods
Blank urine samples (one donor) were spiked with one designer benzodiazepine each, leading to a final concentration of 100 ng/ml. The spiked urine samples were analyzed on an AU680 analyzer with the CEDIA as well as the HEIA benzodiazepine kit. Samples with a response higher than 150 ng/ml were diluted with blank urine (final concentration for second analysis: 10, 20 or 50 ng/ml). Additionally, higher concentrations (200, 500 or 1000 ng/ml) of samples with an initial response lower than 75 ng/ml were analyzed.

Results
The cross-reactivity of the designer benzodiazepines ranged for the CEDIA from 28% (nifoxipam) to 200% (adinazolam) and for the HEIA from 42% (meclonazepam) to 550% (dicyclazepam). The CEDIA showed a cross-reactivity of over 100 % for most of the compounds tested (22 out of 29), compared to 19 out of 29 tested compounds when applying the HEIA.

Tofisopam, a 2,3-benzodiazepine, was the only tested compound which did not show any cross-reactivity in both assays. This drug did also not show any significant cross-reactivity in any other ‘non-benzodiazepine’ immunochemical assay kits (e.g. cocaine, methadone, and tricyclic antidepressants) which were additionally applied for this compound. Furthermore, the HEIA did not show any detectable cross-reactivity at the highest concentration measured (1000 ng/ml) for five of the designer benzodiazepines while this was not the case for the CEDIA.

Discussion
Both immunochemical assays showed a good cross-reactivity for most of the compounds tested.

One pronounced difference that could be observed between the two assays was that, with the exemption of fluclozolam, there is a lack of a sufficient cross-reactivity of the HEIA for thienotriazolodiazepine- and thienodiazepine-compounds (etizolam, metizolam, deschloroetizolam, and bentazepam).

Conclusions
The CEDIA and the HEIA benzodiazepine kits seem suitable for an initial screening for designer benzodiazepines in urine as they both show good cross-reactivity for most of the tested compounds. Uptake of designer benzodiazepines such as metizolam and deschloroetizolam might however be missed when applying the HEIA assay.

Literature:
Abstract ID  547  
Toxicological drug screening in human serum by HPLC with photodiode-array detection: Application in clinical and forensic toxicology.  
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Aim  
In clinical and forensic toxicology, general unknown screening of drugs is usually used, especially when dealing with comatose patients with toxicological etiology or sudden death syndrome. The aim of this study is to present a toxicological drug screening method in human serum based on high-performance liquid chromatography with diode-array detection (HPLC-DAD) developed in our department and estimate its interest in the clinical and forensic toxicology field.

Methods  
In this detection method, a single-step liquid-liquid extraction procedure was optimized for isolation from serum of a broad range of acidic, neutral and basic drugs using dichloromethane/n-heptane/2-propanol (60:26:14 v/v/v) and a pH 9.5 ammonium chloride buffer solution. The analysis of the extracts (identification) were performed using a Perkin Elmer® device with an FLEXAR® PDA (PHOTODIODE ARRAY) LC Detector, a ZORBAX Extend-C 18 column (5 μm, 150 × 4.6 mm) with acetonitrile/phosphate buffer pH 2.6 as mobile phase (flow rate 1.3 mL/min). Full UV spectra from 200 to 400 nm (resolution 1nm) are recorded on-line during the 30min chromatographic run. A self-made HPLC-DAD library with spectral and chromatographic parameters of pharmaceutical and psychotropic drugs was built. The compounds of the library are identified by matching their analytical data (retention times, UV spectrum, wavelengths of the maxima) with the data of Clarke’s analysis of Drugs and Poisons.

Results  
This validated method allow the simultaneous detection of 23 pharmaceutical and psychotropic drugs of interest in the urgent toxicological screenings in our laboratory (benzodiazepines, antidepressant drugs, analgesics, antiepileptic drugs and other psychotropic drugs). The extraction recovery was between 35% and 104%. Specificity was checked for the 23 compounds. The limits of detection (LOD) were between 0.05 and 2 µg mL⁻¹, the LOD had been compared to therapeutic and toxic concentrations usually found in clinical toxicology. However, we have to check if this method did fit to the forensic toxicology purposes. Unknown drugs in patient’s serum samples from acute drug poisoning cases are identified by matching their analytical data (retention times and ultraviolet spectra) with reference compounds previously recorded in the library.

Discussion  
The method gave pretty good qualitative results and overall, the results indicate that the extraction method is well adapted for clinical toxicology purposes. However, specific cases of drug identification in a patient’s serum highlighted the advantages and limitations of this method. The main difficulty was the identification of metabolites if they have very similar spectra compared to the parent compound and if no standards are available.

Conclusions  
The procedure used is simple, specific and relatively inexpensive and it could be used in first intention for urgent toxicological screenings. Implementing it in our laboratory allowed us to create a method of comprehensive screening of drugs frequently implicated in clinical toxicology. However, a further confirmations and development of a comprehensive general unknown screening of drugs and toxic compounds, and a quantification techniques remain necessary for the forensic toxicology purposes.
Abstract ID 548
Optimization and matrix effect assessment of a fast HF-LPME methods for the determination of DMT and harmala alkaloids in urine.
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Aim
The ayahuasca tea is an indigenous hallucinogenic beverage used for shamanic and spiritual purposes, prepared by decoction of different Amazonian plants containing dimethyltryptamine (DMT) and harmala alkaloids (harmine, harmaline and tetrahydroharmine). Since the therapeutic potential of the tea is being largely studied in the recent years, mainly for the treatment of psychiatric disorders, such as addiction and depression, the determination of the components in human and animal matrices is mostly required. Sample preparation methods reported in the literature show the use of large amounts of toxic solvents for protein precipitation, dilution or liquid-liquid extraction (LLE). In this context, hollow fiber liquid phase microextraction (HF-LPME) brings a greener and time saving alternative with the use of extremely low solvent volumes (µL). The present study aims to develop, optimize and evaluate matrix effect, recovery and process efficiency of a HF-LPME method for the determination of DMT, harmine (HRM), harmaline (HRL) and tetrahydroarmine (THH) in human urine using ultra high-performance liquid chromatography coupled to mass spectrometry (UPLC-MS/MS).

Methods
A fractional factorial design with resolution V (25-1) was used to screen significant variables amongst previous stablished ones (buffer pH, buffer volume, stirring time, stirring rate and solvent). Afterwards, a Box-Behnken design was performed to optimize the factors that proved to be important in the first step. Once the factors had been stablished and optimized, limits of detection and quantification were defined as the lowest concentration detected (LoD) or quantified (LoQ) within 20% or 15% RSD, respectively. Matrix effect (ME), recovery (RE) and process efficiency (PE) were obtained according to Matuszewski, Constanzer and Chavez-Eng (2003) protocols in three different concentrations: 15 (LQC), 170 (MQC) and 320 ng/mL (HQC); each concentration was performed in six replicates, each replicate obtained from a different subject.

Results
Buffer pH (9.5 to 10.5), buffer volume (100 to 500 µL), stirring time (5 to 15 minutes), stirring rate (1200 to 2400 rpm) and solvent type (octanol and decanol) were carefully evaluated during optimization, resulting in the following extraction procedure: an aliquot of 500 µL of urine is added into a 2 mL tube containing 80 mg of NaCl; 50 µL of internal standard (DMT-d6 1 µg/mL) is introduced into the mixture followed by 300 µL of 0.1 M carbonate - bicarbonate buffer pH 10.5. A 8 cm polypropylene hollow fiber segment previously impregnated with decanol and filled with 2 mM ammonium formate buffer with 0.1% formic acid (mobile phase A) is introduced into the system which is stirred during 5 minutes at 2400 rpm. The acceptor phase is collected, dried under nitrogen stream at 50 °C and resuspended with 50 µL of mobile phase A before injection (2 µL).

Discussion
LoD was 1.0 ng/mL for DMT and 2.0 ng/mL for the harmala alkaloids. LoQ was 5.0 ng/mL for all analytes. ME varied from 84.3% (HRM, HQC) to 136.6% (HRM, LQC), RE ranged from 57.6% (HRM, LQC) to 205.4% (DMT, MQC) and PE ranged from 78.6% (HRM, LQC) to 253.1% (DMT, MQC).

Conclusions
A potential simple, time saving and green alternative for the analysis of DMT and harmala alkaloids in human urine was developed and optimized. This sample preparation approach has appropriate LoD and LoQ values. The method shows good recovery, matrix effect and process efficiency for harmala alkaloids and unusual high values were found for DMT, which must be further evaluated. Nevertheless, this HF-LPME method will be subjected to complete validation according to current guidelines.
Abstract ID 551
Metabolic profiling of mice urine and cerebral tissue extracts for the discovery of novel biomarkers of ethanol toxicity and abuse.
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Aim
The inability to accurately assess patterns of alcohol misuse presents a significant barrier to the diagnosis and treatment of hazardous alcohol abuse but also to forensic investigations related to these cases. The goal of this project was to study the ethanol-induced biochemical perturbations by the untargeted metabolic profiling of mice urine and cerebral tissue samples thus aiding to the elucidation of the biochemical mechanisms of alcohol toxicity and to the discovery of new biomarkers of alcohol toxicity and abuse. Here, the long-term, as well as the short-term alcohol exposure were evaluated and biomarkers related to ethanol toxicity were deciphered. In recent years, metabolomics-based discovered biomarkers have shed light on various diseases biochemistry but have also attracted interest from other areas such as those with focus on forensic applications.

Methods
The long-term experiment (8 weeks) included 36 C57BL/6 mice (8-10 weeks old) of both genders separated into control and ad libitum fed by a Lieber-DeCarli ethanol diet group for 8 weeks containing daily 5% alcohol 99%. The short-term animal experiment was conducted in male mice for 11 days. In addition, a single dose by oral gavage of 25% alcohol, at the 5th day of the intervention and at the last day of experiment 6 hours before sacrifice were administrated. Urine samples were collected in two time points, while tissues were collected post mortem. The animal experiment was conducted in agreement with the current national and European legislation (Ν. 2015/1992, ΠΔ 56/2013, European guideline 2010/63). Targeted and untargeted metabolomics were applied for urine and cerebral tissue samples, respectively. A targeted HILIC-MS/MS method was performed for the determination of 101 polar metabolites (sugars, amino acids, organic acids, amines, etc) in a single run of 40 min, while an HRAM Q_TOF untargeted analysis was conducted to investigate the distribution of several lipid classes, which have been considered as potential biomarkers for excess ethanol intake. The collected data were further processed using Waters MassLynx and TargetLynx, MS-Dial and XCMS online for metabolite identification. Multivariate (SIMCA 13.0) and univariate statistics were performed to evaluate mice metabolic profiles of the study biospecimens.

Results
Phenylalanine, tyrosine and tryptophan, taurine and hypotaurine and arginine and proline metabolism were found to be differentiated in urine samples of chronic alcohol consumption mice group. Furthermore, Acetyl-carnitine, some PLs, some CERS, Adenine, Adenosine were found to be decreased, while Arginine, Glutamic acid, Glutamine, Aspartic acid, Leucine, Phenylalanine, Proline, Tyramine, Hypoxanthine, Xanthine, Oxoprolinie, Choline, Creatinine, Docosahexaenoic acid (DHA), were found to be increased, in brain extracts of chronic alcohol consumption mice group based on the untargeted analysis. The obtained data from brain extracts of the short-term animal experiment indicated decreased Arachidonic acid (ARA), Docosahexaenoic acid (DHA), Ethyl hexadecanoate, Glycerophosphocholine, CDP-choline, some PLs, Adenine, Adenosine, Cytidine, Creatine, Creatinine, Valine, Taurine.

Discussion
Both applied acute and chronic intervention which led to mild steatosis revealed significant metabolic perturbations which were reflected in urine samples but also in post mortem brain tissues. Study of the most selective ethanol induced metabolic perturbations would allow the discovery of valid biomarkers of ethanol chronic or acute toxicity and misuse which can confirm ethanol involvement in cases of forensic interest.

Conclusions
Metabolomics in both untargeted and targeted approach were proven to be a useful tool in order to elucidate the toxic effect of alcohol consumption and to promote the discovery of novel biomarkers with special focus on forensic applications.
**Abstract ID 19**

**Methyl 4-hydroxybutyrate and ethyl 4-hydroxybutyrate as potential markers for simultaneous consumption of GHB/GBL and alcohol.**

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**Aim**

Due to the rapid occurrence of behavioral alteration effects by reason of high dosed GHB intake (e.g. loss of physical control, unconsciousness) and frequently arising anterograde amnesia of the victims, GHB and its precursors are misused as so-called “knock out drugs” along with, among other crimes, drug-facilitated-sexual-assaults also known as “date rapes”. In addition to the anterograde amnesia, the short detection window (max. 6 - 8 h in blood and max. 10 -12 h in urine) and major inter- and intra-individual variations of endogenous GHB concentrations complicate the analytical proof of an exogenous GHB/GBL administration.

We searched for an alternative way to prove an exogenous GHB/GBL administration via detection of methyl- and ethyl-4-hydroxybutyrate which could arise in alcoholic solutions after spiking with GHB/GBL.

**Methods**

A LC-MS/MS method was developed and validated to quantitatively determine methyl- and ethyl-4-hydroxybutyrate in alcoholic beverages (LoD: 5.79 ng/mL / 3.35 ng/mL).

**Results**

A sample collective of alcoholic beverages (n=47) revealed natural occurring amounts of ethyl 4-hydroxybutyrate in a range from 74 – 3980 ng/mL mainly in wine samples. Nearly no ethyl-4-hydroxybutyrate was observable for spirits and liqueurs and no methyl-4-hydroxybutyrate was detectable at all. A moderate correlation was shown for ethyl-4-hydroxybutyrate concentration and the pH-value as well as the GHB concentration (<LoQ - 11.46 µg/mL). No correlation was detectable with the alcohol content.

A voluntary intake experiment (n=1) revealed no observable GHB-ester concentrations in blood and urine after administration of 750 mL wine with high natural ethyl-4-hydroxybutyrate amounts (2010 ng/mL). In an additional experiment, simulating a DFC case, high amounts of GBL were spiked to an ethanol/water mixture (c(GBL)=18 mg/ml; 15 %vol ethanol) to observe ester production within one hour: no GHB-ester concentration above the LoD could be observed. This indicates that a commonly consumed alcoholic beverage spiked with GHB or GBL would, in comparison to the naturally occurring ethyl-4-hydroxybutyrate amounts, not contain a considerable amount of GHB-ester one hour after GHB/GBL addition.

**Conclusions**

Consequently, it could be assumed that methyl- and ethyl-4-hydroxybutyrate are not useful as markers for the co-consumption of GHB/GBL and alcohol.
New psychoactive substances in Turkey: Narcotics cases assessed by the Council of Forensic Medicine between 2016 and 2017 in Ankara, Turkey.

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Abstract ID 112

New psychoactive substances in Turkey: Narcotics cases assessed by the Council of Forensic Medicine between 2016 and 2017 in Ankara, Turkey.

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Aim

According to the European Drug Report 2018, synthetic cannabinoids (45%) and synthetic cathinones (33%) constituted the most used and diversified NPS groups in Europe in 2017. Although there was a recent increase in information about the use and diversity of synthetic cannabinoids in Turkey, there is no information about the availability, diversity, and use of all types of NPS. This study contributes to the information about the prevalence of NPS use in Turkey and provides the first results on the issue.

Methods

The products containing the NPS (tablets, herbals, different kind powders, blotter stamps) that were seized by the police. Narcotics reports were generated for products containing NPS. In all, 1357 narcotic reports obtained and reviewed between January 01, 2016 and December 31, 2017 in the Ankara Narcotic Department of the Council of Forensic Medicine at the request of the judicial authorities were retrospectively reviewed for NPS. This study was performed by permission of the Presidency of Scientific Board of Council of Forensic Medicine. The samples were dissolved in methanol to injection into the GC–MS system. Cayman, Nist, SWGDrug and Wiley libraries were used. In cases in which the probability of scanning the substance in the library scan of GC–MS is below 90% or any suspicion, confirmation was performed. Thermo Q Exactive Plus Quadrupole-Orbitrap LC–MS/MS System and Liquid chromatography tandem mass spectrometry (LC-MS/MS) were used for confirmation.

Results

For data collection, a total of 1357 narcotic cases were investigated during a 2-year period. Among the cases, 29 different NPS compounds were detected. The NPS content of the products were classified as synthetic cannabinoids, cathinones, tryptamines, and phenylethylamines. The NPS compounds detected in the study according to this classification are given below.


Cathinones: 2-CEC (2-Chloroethcathinone), 4-CEC (4-Chloroethcathinone), 4F-a-PHP (4-F-a-pyrrrolidinohexiophenone), 4-MMC (4-Methylmethcathinone), a-PVP(a-pyrrolidinopen-tiophenone), 4-chloromethcathinone (4-CMC), 3-CMC 4- methyl-N, N-dimethylcathinone and 3-MMC. α-PVP, also known as ‘Flakka’, is an emerging product in Turkey. This is the first report of its presence and use in Turkey, along with the first report of five other synthetic cathinones. Tryptamines are among the important substances detected in this study. Interestingly, a member of this group, 5-MeO-MiPT, had the second highest amount confiscated after 5-F-ADB, a synthetic cannabinoid. Detecting three important members of the tryptamine group in the study indicates that there is a demand for this group in Turkey. This is a clear indicator of the need for extra precautions because there is no information on their use and diversity in Turkey. Thus, the results obtained in this study should alert the Ministry of Health and other related institutions.

Only two types of phenethylamines, 2C-C and 2C-I, were detected. These two active substances were simultaneously detected in 9960 blotter stamps. This capture rate is the highest in Turkey so far. The capture of these two phenethylamine samples in Turkey is the most important indicator of the new search for and use of NPS in Turkey.

Conclusions

NPS represent a broad class of pharmacologically active compounds that demonstrate various effects through different mechanisms. Not fully understanding the effects of NPS, their absence from regulations, the presence of multiple NPS in one product, their increasing abuse, and not knowing which substance was used render their use extremely dangerous. In light of these problems, NPS-containing products are the most problematic narcotic substances. Additional studies are needed to assess their toxicity to overcome the health issues related to the abuse of NPS and revise public health policies.
Abstract ID 141
Detection of drugs used in Drug Facilitated Sexual Assault for five years (2014-2018) in Korea.

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Aim
Drug-facilitated crime (DFC) is a crime in the course of which the victim is administered a drug or other incapacitating substance in order to affect control over their actions, decision-making capacities or physical capabilities. It includes crimes such as sexual assaults, robberies and gambling, and whilst most commonly known form is a drug facilitated rape or a drug facilitated sexual assault (DFSA). With the recent involvement of a famous Korean celebrity in DFSA, it has emerged as the biggest issue in Korean society. The National Forensic Service (NFS) is a national institute that supports law enforcement agencies to resolve various crimes by analyzing evidences scientifically. Including the main office at Wonju, there are five local institutes (Seoul, Busan, Daegu, Daejeon, and Gwangju). In order to investigate the current status of DFSA in Korea, we reviewed DFSA cases of NFS.

Methods
Urine and/or bloods from victims of sexual assault were commissioned by the police to the NFS for drug analysis. Gas chromatography/mass spectrometry (GC/MS) and Liquid chromatography/tandem mass spectrometry (LC/MS/MS) after solid-phase extraction were performed for the detection of drugs in blood and urine samples. Analytical results in DFSA were investigated by the laboratory information management system of NFS for five years (2014-2018). Mainly, the total count of DFSA cases, drug detection rate, types of drugs and frequency of use were investigated.

Results
In the recent five years, the number of DFSA case increased every year, from 559 cases in 2014 to 1459 cases in 2018, over 161% from 2014. The number of the incidents in Seoul institute was shown the highest, occupying 60% of the total, and followed by Wonju (11%), Busan (10%), Daegu (9%), Daejeon (5%), and Gwangju (5%). Since Seoul is the capital of Korea and has a large population in the metropolitan areas, thus the number of case and the population was expected to have higher correlation. The highest drug positive rate detected in total was observed in 2014 at 33%, while an average positive rate was 27% for five years. The total 114 types of drugs were detected and the most frequently used in the crimes drug was Zolpidem with 429 times for five years, occupying for 16% of all drugs. Next, benzodiazepines such as diazepam, lorazepam, and alprazolam were detected with 178, 144 and 113 times, respectively. Meanwhile, illegal drugs were also found, which includes amphetamines (51 times), cannabis (12 times), and ketamine (7 times).

Discussion

Conclusions
DFSA in Korea has increased every year and the types of drugs used in the crimes also have become diversified. Though most of these drugs were hypnotics and sedatives, illegal drugs were also detected at a relatively high rate. This analysis will be an useful evidence proving the need for well-functioning framework preventing DFSA and consequently support law enforcement authority to establish effective legal system.
Abstract ID 233
Difficulties in interpretation of forensic toxicology results when suspected intoxication is related to gamma-hydroxybutyrate and analogues.
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Aim
This work describes difficulties in interpretation of gamma-hydroxybutyrate (GHB) positive results based on a case of acute mixed poisoning with GHB and ethanol with a fatal outcome (brain death), methodology of toxicological analyses of ante-mortem and post-mortem biological material, as well as a syringe found in the place of crime filled with transparent liquid. In December 2014, at about 04:10 a.m., physicians from emergency medical services were providing medical assistance to a woman and two men who, as became known from an interview, ingested an unknown amount of GHB for recreational purposes. The woman and one of the men left the hospital shortly after being provided with medical assistance in the hospital emergency department. During an interview concerning their medical history, both of them reported that in contrast to their friend, they self-induced vomiting soon after ingesting GHB, fearing its toxic effects. The man who ingested the entire dose of the substance was admitted to the intensive care unit after two attempts at cardiopulmonary resuscitation on the street. After 5-th day of treatment the adjudication committee pronounced brain death, and the patient’s organs (heart and kidneys) were recovered for transplantation.

Methods
Routine immunological tests of the ante-mortem urine sample collected from the patient revealed the presence of cannabinoids. Analysis of the post-mortem urine sample detected cannabinoids, benzodiazepines and barbiturates. Hair samples were not collected, so the examination towards possible addiction to psychoactive substances has not been verified. Since the patient was treated with Relanium (diazepam) and Thiopental (thiopental) at the hospital, confirmation of the presence of medicines from the group of benzodiazepines and barbiturates in biological material was discontinued. To confirm the presence of cannabinoids liquid-liquid extraction were applied and the resulting extracts were analysed by gas chromatography combined with mass spectrometer (GC/MS) in the selected ion monitoring (SIM) mode using negative chemical ionisation for delta-9-tetrahydrocannabinol (THC) confirmation and using electron ionisation for 11-nor-9-carboxy-delta-9-tetrahydrocannabinol acid (THC-COOH) determination. In order to detect ethyl alcohol and determine its content ante-mortem blood sample were analysed by routine headspace gas chromatography with flame ionization detector. Further analyses were carried out to confirm the presence of GHB. Samples of biological material were collected for extraction with methanol, derivatised by adding BSTFA:TMCS (99:1) and analysed in the GC/MS-SIM. In the course of the instrumental analysis, the mass of ions characteristic for GHB (m/z: 233, 117, 147) and for GHB-D6 (239, 117, 147) was monitored.

Results
In toxicological analysis of urine sample fatal concentration of GHB was detected (3000 µg/mL). The presence of ethyl alcohol in blood and urine samples (0.60 per mille and 0.74 per mille respectively) were collected from man intoxicated with GHB at 09:08 a.m. (December 2014). The analysis additionally detected psychoactive THC below the quantitation limit and its inactive metabolite THC-COOH in concentrations of 128 ng/mL in blood and 36 ng/mL in urine. Such level may witness the previous ingestion of hashish or marijuana, which however, were not responsible for death. The transparent liquid from the syringe contained GHB, which was identical with the substance detected in the ante-mortem biological material.

Discussion
The presence of ethyl alcohol in ante-mortem blood serum and urine samples collected from the man at 9:08 a.m. (i.e. after about 5 hours after the calling of the police to the place of the incident) on the date of his poisoning in December 2014 indicates the possible combined strong depressive effect of GHB and ethanol on the central nervous system and the respiratory system, which in consequence might have led to cardiac arrest and death, although the ingestion of GHB alone was sufficient to cause the lethal effect, and the ethyl alcohol detected in the victim’s body was not a factor indispensable for sudden cardiac arrest and the man’s death. Detection of psychoactive THC and its non-active metabolite THC-COOH (in concentrations indicating only an earlier ingestion of cannabinoids), had probably not a significant impact on the cause of death due to usually low toxic effect of cannabis in comparison to GHB and alcohol.

Conclusions
According to the medico-legal report drawn up after the inspection and dissection of the corpse, the man’s death was caused by a diffuse, acute, ischaemic brain injury with oedema, complicated by intracranial hypertension and herniation of the cerebellar tonsils into the foramen magnum. Chemical toxicological analyses of ante-mortem samples of biological material detected the psychotropic substance GHB, which was determined in concentrations recorded in the urine for toxic and lethal effect. The presence of ethanol in samples of blood serum and urine indicated a possible strong combined depressive effect of GHB and ethanol on the central nervous system and the respiratory system. The analyses additionally detected psychoactive THC and its inactive metabolite THCCOOH, in concentrations indicating previous ingestion of hashish or marijuana.
Abstract ID 241
Drug-facilitated crimes: 15 years results of French prospective surveys.
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Aim
Drug-facilitated crimes (DFC) is a national program set up by the French Agency for the Safety of Medicines and Health Products (ANSM), under the responsibility of the Paris Addictovigilance Centre, with prospective survey and regular preventive messages for many years. DFC is defined as the concealed or forced administration of psychoactive substances (PAS) to victim for criminal purposes (sexual assault, homicide, robbery...). In this situation, the victim is under influence of PAS consumed unwittingly (chemical submission, CS) while, in cases of chemical vulnerability (CV), PAS (mainly alcohol) are voluntarily consumed by victims, putting them in a more vulnerable state.

Methods
We report the results of French prospective surveys on DFC carried out between 2003 and 2017. Data were collected mainly from forensic toxicological laboratories, then forensic emergency units and more rarely by other structures and the victims themselves. The assessment is based on clinical data and toxicological analysis results in blood and/or urine and/or hair using chromatographic techniques coupled with mass spectrometry.

Results
We analysed 4420 cases files for victims of suspected DFC with an average of 340 reported cases per year. Among these cases, 55% were not enough documented for an accurate diagnosis (lack of adequate toxicological analysis and/or clinical data), while CV represents 26% of cases (1166) and CS/DFC in only 19% of cases (844). The most prevalent victims are female (80%) with mean age of 26.8. Children under 15 and the elderly are also affected particularly. Sexual assault is the main aggression (73%), followed by theft and burglary (16%). Amnesia and vigilance disorders were found in respectively 61% and 16% of CV group and in 51% and 31% of CS group.

Toxicological analyses were performed on blood (51% of victims), urine (43%) and hair (13%). The CV group is divided in two clusters: cluster A (CV-A) with only of non-therapeutic drugs (72%) and cluster B (CV-B) with all kinds of PAS (28%). Alcohol and cannabis intake were involved in 80% and 28% cases, respectively.

In the CS group, children and elderly were mainly victims of mistreatment which is called “chemically beaten subjects” (40% of children). In this group most predominant PAS detected were benzodiazepines and Z-drugs (BZD) (57%), followed by various non-therapeutic substances (16%) and then antihistamines (15%). GHB was detected in only 3% cases. Among BZD, three were more frequently used: clonazepam (21%), zolpidem (18%) and bromazepam (17%).

Discussion
49% of victims consulted in less than 16 hours after the assault, but in 51% of cases blood and urine were sampled later. Nevertheless, appropriate toxicological analyses were performed. Sexual assault remains the main crime and the BZD are the predominate used PAS, while GHB is rarely found despite the appropriate toxicological analysis carried out in due time. We can conclude that the most frequently substances used in the SC are PAS used in the general population and therefore those that are found at home.

Conclusions
DFC unfortunately persists despite regular national information campaigns. The contribution of hair analysis is of considerable importance to establish the accurate diagnosis, that participates in the care of victims. The main obstacle to its use for all victims remains its high cost, that can explain the high proportion of excluded cases. The cases involving GHB are probably underestimated probably because of its illicit drug status and its very short half-life that prevents detection in delayed laboratory samples. Alcohol remains the most important substance involved in the chemical vulnerability.
Abstract ID 311  
**Dangerous online Diazepam counterfeits.**

**Katerina Hajkova**

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**Aim**

The aim of this study was to find out if tablets bought on the dark web as Diazepam contain the substance or not; and if not Diazepam what it could be?

**Methods**

We bought a package containing 100 tablets of “Diazepam” on the dark web; then we purchased another package for confirmation purposes. The sample preparation technique was based on a dissolution of tablets in methanol, the solutions were diluted with mobile phase prior to the LC-HRMS analysis. LC-MS determination of the analytes was based on the application of Zorbax Eclipse Plus C18 column (100 × 2.1 mm i.d., 1.8 µm) with pre-column and gradient elution with A: 5 mmol/L ammonium formate containing 0.01% formic acid and B: methanol containing 0.01% formic acid. The LC system was hyphenated with a 6550 iFunnel Q-TOF MS in positive electrospray mode. We used non-target analysis with auto MS/MS acquisition in MassHunter workstation. Data were evaluated owing to MassHunter with integrated PCDL library and online free databases.

**Results**

All tablets bought on the dark web as Diazepam turned out to be counterfeits after analysing using LC-HRMS method. We applied our non-target method for common fakes and drugs, for which an in-house MS/MS library was developed. On analysing for common “Diazepam” we found the tablets to contain Etizolam instead; not known in our library so far. Online search of free databases as DrugBank, Chemspider, PubChem etc., we were able to compare our findings and reached a conclusion that it was Etizolam as this matched results from other databases. Etizolam was identified owing to accurate mass, isotopic pattern and MS/MS spectra at 10, 20, and 40 eV. An analytical standard was purchased for confirmation purposes.

**Discussion**

Benzos are commonly sought after psychoactive drugs and are sold online on illicit drug market. From our analysis, we found Etizolam being sold as Diazepam, leading us to conclude that counterfeits of benzos not easily distinguishable from the original drugs.

**Conclusions**

We detected tablets sold as a Diazepam; the product was barely distinguishable from original Diazepam but contained another active pharmaceutical ingredient (API). Instead of declared Diazepam, we found the tablets to be Etizolam, which is not legally available in most European countries. This kind of near perfect counterfeit can easily mislead, resulting in danger to public and challenges for medical staff to deal with emergencies.
New evidences on natural presence of GHB in common beverages and data interpretation in suspected DFSA cases.

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Aim
GHB, g-hydroxybutyric acid, is considered one of the most diffused rape drugs and its use in drug-facilitated sexual assaults (DFSA) is well documented. It produces disinhibition, amnesia and sex drive; moreover, it is colourless, odourless and water soluble, thus it can be easily added by perpetrators to drinks. GHB is an endogenous substance in human body and it is naturally present in many beverages. In fact, many publications reported GHB in red and white wine, in tonic and lemon tonic water, tequila and other products. This may lead to misinterpretation especially in forensic cases. To the best of our knowledge, no studies have been focused on the natural presence of GHB in the energy drinks. It must be pointed out that energy drinks are often mixed with alcoholic beverages for recreational purposes, then can be easily used to administer GHB to unsuspecting victims. In this research we aimed to collect evidences about GHB presence in several energy drinks, commonly available in Italy, in order to ensure a better interpretation of analytical findings in biological sample from suspected DFSA cases.

Methods
This study is still ongoing and so far, we analysed 10 energy drinks brought from various local stores. Multiple products were analysed of the same drink (e.g. sweetened and diet). After degassification in an ultrasonic bath for 15 min, the samples were extracted by an acidic liquid-liquid extraction with ethyl acetate. The organic mixtures were then derivatized with BSTFA. Deuterated GHB (GHB-d6) was used as internal standard. Analyses were performed by a GC-MS system consisting in an Agilent 7890A GC system equipped with an Agilent 7683B series autosampler and interfaced via electronic impact source to a single quadrupole Agilent 5975C mass spectrometer. The column was an Agilent HP-5 (30 m, 0.32 mm, 0.25 mm). The oven temperature was set initially at 60 °C for 0.5 min and programmed to raise up to 130 °C (increase rate: 10 °C/min) and up to 300 °C (increasing rate: 8 °C/min). Acquisition was in SIM: GHB, 233, 234, 147; GHB-d6, 239, 240 and 147. The limit of detection and the limit of quantification were 1.3 (0.0013 mg/L) and 2.5 ng/mL (0.0025 mg/L), respectively.

Results
Currently, 10 energy drinks have been analysed and we aim to increase this number to at least 30 different products, even from different Italian cities. GHB was found in all the samples and concentrations ranged from 119 to 139 ng/mL (0.119-0.139 mg/L).

Discussion
GHB is naturally present in the energy drinks we analysed so far. Concentrations were similar to the ones described in literature for tonic water (0.13-0.18 mg/L). Anyway, those GHB amounts are far lower than the GHB effective doses. It is known that they range from 0.5 g to > 4 g (unconsciousness); 1-1.5 g is considered as recreational dose. So, for example, the lowest active 0.5 g dose dissolved in 250 mL (reaching a 2000 mg/L concentration) is about two orders of magnitude higher than the natural occurring GHB levels. For this reason, discrimination between endogenous or added GHB is now to be considered an almost solved problem.

Conclusions
This study is still ongoing thus we are not able to achieve a final conclusion. Anyway, in this preliminary phase, we observed the GHB presence in 10 energy drinks, providing a first evidence of its natural presence in this kind of products. Currently, we are increasing the number and type of beverages and we planned to analysed up to other 20 products in the next two months.
Abstract ID 345
Development and validation of a simple and quick method for determination of 521 psychoactive substances, drugs and their metabolites from blood by LC-MS/MS.
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Aim
The popularity of recreational use of new psychoactive substances (NPS) has been systematically increasing in recent years. This phenomenon is intensifying due to the change in the model of life and the availability of new methods of communication and trade. The widespread intake of psychoactive compounds leads to serious threats to the life and health of people who are not only enjoying but also poses a serious threat to outsiders and society. The model of toxicological tests established over the decades, based on methodological screening tests confirmed by instrumental studies, becomes ineffective due to the richness and diversity of currently used psychoactive compounds. At present, it is very important to develop and validate methods for determining the largest possible amount of psychoactive compounds and drugs at the lowest possible level of concentrations in the shortest possible time.

Methods
Validation was conducted for the method of multiplex analysis of 549 compounds belonging to different groups: cathinones, synthetic cannabinoids, opiates, amphetamines, benzodiazepines, tricyclic antidepressants and others. Certified analytical standards of individual analytes were used to develop the method. The chromatographic separation conditions and mass spectrometer working conditions were selected for the obtained analytical group. Liquid-liquid extraction was used to isolate analytes from blood samples. Qualitative and quantitative analysis was performed in the MRM pair monitoring mode for every analyte. The analyzes were carried out using a HPLC 2xExion LC AC Pump, Exion LC Degaser, Exion AC Autosampler, Exion LC Column Oven coupled to a Sciex 5500 QTRAP mass spectrometer operated in ESI mode, and a Kinetex C18 100 x 3 mm, 2.6μm chromatographic column as well as a buffered methanol/water mobile phase system.

Results
Validation was carried out in accordance with the SWGTOX guidelines. The following validation parameters were determined: LOD was determined as the lowest calibration standard which exhibited an S/N ratio ≥ 3, while the LOQ was determined to be the lowest calibration standard which exhibited an S/N ratio ≥ 10. LOD ranged between 0,3-1,0 ng/ml and LOQ ranged between 1-10 ng/ml in whole blood. BIAS (+/- 20% from the actual value), precision (+/- 20% from the actual value), reproducibility, specificity and impact of the matrix. A positive validation result was obtained for 521 analytes.

Discussion
The conducted validation allows to establish that the developed method of isolation and determination of 521 psychoactive compounds, including drugs, meets the conditions of the confirmatory method according to SWGTOX.

Conclusions
The validation carried out allowed the introduction of the developed method for routine analyzes regarding, inter alia, drug facilitated crime.
Abstract ID 348

Multiplex determination of psychoactive substances, drugs and their metabolites in urine by LC-MS/MS.

Natalia Galant

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Aim

Urine is one of the most frequently used, non-invasively obtained biological matrices in the field of forensic toxicology. It is a material which not only provides information regarding the general functioning of the body, but also about the recently used psychoactive substances and pharmaceuticals - some of them can be detected even after several days of intake. In the urine there are both primary forms of psychoactive substances and their metabolites. Many of them are excreted in the form of glucuronides. It is thanks to the above urinary properties that analysis of urine samples is used in cases related to drug-facilitates crime.

Methods

Urine samples were prepared using two methods: direct liquid-liquid extraction and extraction with an organic solvent preceded by enzymatic hydrolysis. 549 analytes were validated according to the SWGTOX guidelines. Urine samples without psychoactive substances were used for the validation experiment. Six replicates were made for each level of fortification. Compounds of various chemical structure are within the scope of the method: among others amphetamines, cathinones, opioids, THC and THCCOOH as well as synthetic cannabinoids, benzodiazepines, tricyclic antidepressants, etc. Two MRM pairs were selected for each analyte according to the valid mass spectrometry standards and the spectrometric analysis parameters were optimized, such as: declustering potential (DP), Entrance Potential (EP), Collision energy (CE), Collision Cell Exit Potential (CXP). Analysis was performed using liquid chromatography (HPLC: 2x ExionLC AC Pump ExionLC Degaser, ExionLC Autosampler AC, AC ExionLC Column Oven, column chromatography Kinetex C18 100 x 3 mm, 2.6μm; mobile phase in a buffered water/methanol system) coupled with the quadrupole mass spectrometer AB SCIEX 5500 QTRAP operated in ESI mode.

Results

Among the 549 compounds subjected to validation, 517 analytes meet the criteria recommended by the United Nations Office on Drugs and Crime and SWGTOX: BIAS and precision did not exceed 20% at the each concentration; the regression coefficient of the calibration curve ≥ 0.99. The range of quantification for most analytes is equal to 10 - 5000 ng/ml of urine, whereas for cannabinoids: 1 - 1000 ng/ml of urine. The range of LOD is equal to 0.3 - 1.0 ng/ml.

Discussion

The developed method allows unambiguous identification and quantification of over 500 psychoactive substances in urine samples by means of liquid chromatography coupled with quadrupole mass spectrometry. The validation process confirmed the compliance with validation criteria for confirmatory methods recommended by SWGTOX. The method has been verified in inter-laboratory proficiency tests with a positive result. The use of this method was checked in a routine laboratory analysis.

Conclusions

Due to the presence of metabolites of psychoactive substances among the analyzed substances, the developed method is an excellent tool for analyzing urine samples related to drug-related crimes. The wide range of analyzed substances and the possibility of further development of the method are its additional advantage. Fully validated, compliant with the SWGTOX criteria and confirmed by proficiency testing, the method has been successfully introduced into routine analyzes in our laboratory.
Soaked quality control samples for hair testing.

Marie Nielsen

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Abstract ID 402

Soaked quality control samples for hair testing.

Marie Nielsen

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Aim

In this study, we investigated if drug-free hair exposed to reference standard solution is useful for quality assurance in hair testing, when certified reference materials containing the relevant substances are not available.

Methods

Drug-free hair was soaked with aqueous standard solution (1 mg/L) containing 29 pharmaceuticals and illicit drugs for one hour with constant shaking. The standard solution was discarded and the soaked quality control (QC) sample was briefly washed with methanol. Aliquots of soaked QC were washed with six commonly used washing procedures involving one time with 2-propanol or methanol, three times with water, 10 mM phosphate (pH 6.0) or 10 mM ammonium acetate (pH 5.5), and finally one time with 2-propanol or methanol. Washed QCs and all wash residues were analysed with validated UPLC–MS/MS methods involving pulverization of 10 mg hair and incubation in a mixture of methanol:acetone:ammonium formate (pH 5.3; 2 mM) (25:29:46, v/v/v) at 37 °C for 18 hours. Additional, two other QCs were prepared at different levels using 0.01 and 0.10 mg/L standard solutions, termed QC1 and QC2, respectively. These QCs were monitored in our routine hair analysis of drug-rape investigations in 17 series in a one-year period, to follow the daily performance of our methods and to estimate the long-term precision.

Results

All tested drugs were detectable in soaked hair following one hour of exposure in aqueous standard solution. The incorporated amount of an unwashed aliquot ranged from 0.27 to 4.5 ng/mg depending on the analytes. Following the six different washing procedures, 27–70% of the incorporated amount remained in the hair indicating that the drugs were incorporated into the hair cells in some extent and not only deposited on the surface of the hair. The standard deviations of concentrations in the six aliquots of washed QCs were below 25%, except for three analytes. Thus, only small differences in washing efficiency were observed between the six different washing procedures. For all analytes, the concentration in the aqueous wash residues decreased with increasing number of washing steps. In the last aqueous wash residues the concentrations accounted for 6–27% (average: 12%) of the amount in the washed QCs.

Following routine analysis of soaked QCs at two levels, the soaked QCs were proven to be homogenous within a batch with method imprecision below 20% (range: 5–20% for all analytes, mean: 12%) at both levels. The incorporated amount ranged from 0.002 to 0.026 ng/mg in QC1, and 0.011 to 0.25 ng/mg in QC2, i.e. approximately a factor of 9 between the levels. Thus, as good correlation between the incorporated amount and the concentration in the standard soaking solution was observed. No decline in drug concentration was observed over time indicating that the analytes were stable in soaked QCs stored dark at room temperature for one year.

Discussion

Since it was not possible to remove the entire amount of incorporated drug with the applied washing procedures, this indicates that the analytes were to some extent migrated into the hair cells and not only deposited on the surface of the hair. Thus, swelling agents such as water and methanol may allow drugs to diffuse from the solution into the hair cells similar to the incorporation of drugs from sebum and sweat bathing the growing hair.

A major advantage is that soaked QCs can be produced with hundreds of substances in relevant concentrations in large quantities within a few hours of soaking. Thus, the quality of a broad range of target drugs can easily be monitored using these soaked QCs. Besides quality assurance, soaked QCs are also useful for method development and validation, where they e.g. can be used to evaluate extraction efficiency and method imprecision.

A limitation using soaked QCs for quality assurance is that the analytes are not incorporated into the hair cells in the same manner as the drug incorporation into growing hair from the blood after ingestion. Therefore, differences in the degree of extraction of the substances may occur from authentic hair samples and soaked QCs. Furthermore, soaked QCs is not suitable for evaluation of accuracy, since no true values are accessible. Instead, the accuracy of the method should be evaluated by analysing certified reference material and by participation in proficiency program testing.

Conclusions

This study demonstrated the applicability of using soaked QCs to evaluate method imprecision and to monitor the daily performance of multi-target methods. The soaked QCs were proven to be homogeneous within a batch and stable within a year, and the method was proven to be reproducible for all target analytes with method imprecision less than 20% at both low and high levels. Based on these results it was concluded, that soaked QCs are an excellent tool for daily control of the assay performance as well as for method development and validation of multi-target methods.
Abstract ID 417
HPLC-MS/MS method developed for the determination of 4-FMA in rat plasma.
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Aim
Amphetamine-type-stimulants (ATS) are the second most frequent group of illicit drugs used worldwide, and 4-fluorine methamphetamine, a new psychoactive substance with ATS-like properties, shows more progressive popularization in recent years. To develop and fully validate a high-performance liquid chromatography-tandem mass spectrometry method for the quantitative determination of 4-Fluoromethamphetamine in rat plasma. It will provide a methodological basis for the study of the toxicokinetics of the new psychoactive substance 4-FMA in rats.

Methods
Protein in plasma sample spiked with internal standard methamphetamine (MA) was precipitated with methanol and the supernatant obtained after centrifugation was analyzed by HPLC-MS/MS. The processed samples were separated on Agilent Poroshell 120 EC-C18 column (50 mm*3.0 mm, 2.7 μm). And the mobile phase was water containing 0.1% formic acid and acetonitrile containing 0.1% formic acid(85: 15, V/V) at a flow rate of 0.4 mL/min. The multiple reaction monitoring (MRM) mode was performed and ionization was achieved using electrospray in positive ionization mode (ESI+). For quantification, the protonated molecules of the target analytes with their accurate masses were used, and the MRM transition was: m/z 168.1>109*,168.1>137.0 (4-FMA), m/z 150.1>91.1*, 150.1>118.7 (IS MA).

Results
The method was linear in the range of 5 to 1000 ng/mL (r>0.999). The limit of detection was 3 ng/mL and the limit of quantification was 5 ng/mL. The accuracy (relative error) was less than 5% and the precision of inter-day and intra-day (relative standard deviation) was less than 9%. More than 90% extraction recovery was obtained and less than 8% relative matrix effects were observed for the sample. The sample determination was completed within 2.5 min.

Discussion

Conclusions
An accurate, rapid, simple and sensitive ultra performance liquid chromatography-tandem mass spectrometry method was developed and fully validated for the quantitative determination of 4-Fluoromethamphetamine in rat plasma, which can be applied to the toxicokinetics study subsequently.
Abstract ID 39
Development and Validation of Multi-residue Screening Method for 439 Pesticides in Soft Drink Using LC-MS/MS and GC-MS/MS.
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Aim
When pesticide poisoning occurs in occupational, homicidal or suicidal cases, it is very important to identify rapidly the unknown toxicant and determine its concentration for detoxification treatment or verification of poisoning. Therefore, the establishment of an accurate, precise, rapid and simultaneous multi-residue pesticide analytical method is critically required.

Methods
In this study, an effective and simultaneous multi-residue screening method for the determination of 439 pesticides in soft drink developed using LC-MS/MS (SHIMADZU LCMS-8060) and GC-MS/MS (SHIMADZU GCMS-8040) for forensic and toxicological investigation. The multiple reaction monitoring was optimized with positive/negative ionization mode on LC-MS/MS and GC-MS/MS. In case of soft drink, limit of quantitation (LOQ) was 10 ng/mL for 98.6% of all target analytes. To optimize the sample preparation, all target compounds were spiked in 10 mL of soft drink, extracted with acetonitrile (10 mL), treated with magnesium sulfate (4 g), sodium chloride (1 g), sodium citrate tribasic dehydrate (1 g) and sodium citrate dibasic sesquihydrate (0.5 g) in 50 ml conical tube, and the extracts were centrifuged. And then the extracts (4 μL) were analyzed directly using LC-MS/MS, and the extracts (2 μL) were analyzed directly using GC-MS/MS without further clean-up steps to prevent the loss of labile target analytes and minimize the analysis time.

Results
In the recovery tests at 10 and 50 ng/mL the total average recoveries of all target compounds were from 96.6 to 97.5% (CV≤20%) at two levels.

Discussion
The correlation coefficients (r2) of calibration curves using procedure standard solutions were ≥0.99 for most of target compounds. The averages of matrix effects were 56.0% compounds of all target analytes were included within ±20% of matrix effect in soft drink.

Conclusions
In conclusion, the established analytical methods in this study can be sufficiently applied at hospitals and forensic facilities in need for the monitoring of multi pesticide residues in soft drink samples.

Abstract ID 288
Evaluating some biological parameters as indicators of toxicity as a result of exposure to gasoline in workers at gas stations in Damascus.
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Aim
Gasoline is a blend of organic compounds used in internal combustion engines. Gasoline-station attendants are exposed to gasoline vapors, which pose a potentially mutagenic risk. According to the International Agency for Research on Cancer, exposure to gasoline is possibly carcinogenic to humans.

The aim of this study is to evaluate some biological parameters as indicators of toxicity as a result of exposure to gasoline in workers at gas stations in Damascus.

Methods
Blood samples were collected from exposed workers (n= 30) and healthy controls (n= 30) with no history of occupational exposure, and the following markers of oxidative stress were analysed malondialdehyde (MDA) (OxiSelect™ TBARS Assay Kit (MDA Quantitation), STA-330, Cell Biolabs , usa), advanced oxidation protein products (AOPP) (OxiSelect™ AOPP Assay Kit, STA- 318, Cell Biolabs , usa) , catalase activity (OxiSelect™ Catalase Activity Assay Kit, Colorimetric, STA-341, Cell Biolabs , usa) , CBC, ALT and AST.

Results
We found that the levels of MDA in exposed workers were significantly higher than healthy controls (p < 0.0001). The levels of AOPP in exposed workers were significantly higher than healthy controls (p < 0.044). The levels of catalase activity in exposed workers were significantly higher than healthy controls (p < 0.002).

No statistical significant (p > 0.05) in CBC, ALT and AST.

Discussion
The results from our study showed that chronic gasoline exposure may result in long-lasting oxidative stress, as demonstrated by the presence of statistically significant correlations between gasoline exposure and levels of biomarkers (MDA, AOPPs, Catalase activity).

Conclusions
the early identification of these biomarkers can be very useful in order to promote programs on health protection and prevention for those populations more susceptible to the adverse effects of gasoline exposure.
Abstract ID 349
One-step extraction and detection of 513 drugs from hair by LC-MS/MS.
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Aim
Analysis of psychoactive substances and pharmaceuticals in hair is an increasingly common method used in the diagnosis of addictions and in the field of forensic toxicology. It is a powerful evidence tool, used in many criminal cases, due to the possibility to detect substances up to several months after their potential intake, as a result of their incorporation into the hair structure, which is not possible with other biological matrices such as blood or urine. Analyses are carried out on specific sections of hair from 5-30 mm long or a segmentation analysis of lengths of 5-10 mm is conducted. Based on the results of the analysis of several short segments, a detailed profile of substance intake in the approximate time interval can be created, assuming an average one-centimeter hair growth per month. In the era of new psychoactive substances and drug abuse, it is extremely important to develop a method which allows simultaneous analysis of a wide range of compounds in a single analysis, while the levels of quantification recommended by the Society of Hair Testing are maintained.

Methods
Collected hair with a segment length of 5-30 mm were repeatedly rinsed using isopropanol, water and dichloromethane, dried and pulverized using a ball mill. Extraction was carried out using a one-step solid-liquid method. Validation was carried out for quantitative analysis of 521 analytes following the SWGTOX guidelines. The method included compounds with different properties and chemical structure: e.g. amphetamines, cathinones, opioids, THC and THCCOOH as well as synthetic cannabinoids, benzodiazepines, tricyclic antidepressants, etc. Two MRM pairs were selected for each analyte according to the valid mass spectrometry standards and optimal analysis parameters were determined, such as: DP, EP, CE, CXP. The analyses were carried out using LC-MS/MS (2x Exion LC AC Pump, Exion LC Degaser, Exilon AC Autosampler, Exion LC Column Oven, Kinetex C18 100 x 3 mm, 2.6 μm chromatographic column, mobile phases in buffered water/methanol, QTRAP 5500 system operated in ESI mode).

Results
Among the 521 validated compounds, 513 analytes meet the criteria recommended by the United Nations Office on Drugs and Crime and SWGTOX: BIAS and precision did not exceed 20% at each concentration, while the levels of quantification recommended by the Society of Hair Testing (amphetamines, opioids, methadone - 0.2 ng/mg, cannabinoids 0.05 ng/mg, cocaine - 0.5 ng/mg, selected metabolites - 0.05 ng/mg). In case of some compounds or their groups, levels of quantification lower than recommended were determined, e.g. amphetamine - 0.125 ng/mg or cannabinoids - 0.025 ng/mg.

Discussion
The developed method meets the validation and analytical criteria of recommendations for screening and confirmation methods. Only in case of THC determination with a simultaneous lack of metabolite (THCCOOH), additional confirmatory analyzes are necessary with a level of quantification of 0.2 pg/mg, which requires specific conditions for GC-MS/MS analysis or LC-MS/MS analysis in MS3 mode.

Conclusions
The developed method allows quick analysis in a wide range of analytes. The use of this method in analysis of forensic toxicology samples may contribute to an increase in the number of new psychoactive substances detected and assist the procedural authorities. The above method has been successfully introduced into analyzes in our laboratory and may be extended to include further substances appearing on the black market.
Abstract ID 490

Drug free campus policy in Ege University, Turkey: the importance of drug monitoring stage.

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Aim
Drugs abuse problem has long been a deep concern of the world and our society. Turkey is a transit country between Europe and the Middle East and also represents a large consumer market. Today’s young people face a range of drug temptations never envisioned by earlier generations. According to Turkey Statistics Agency 2018 data, Turkey have 12 million young population. Responding to these risks before they become more difficult problems, one of the goals of the Ege University (EU) Institute on Drug Abuse, Toxicology and Pharmaceutical Science (BATI) is working for increase the awareness for drug abuse and implements preventive approaches by “Drug Free Campus and Workplace” policy for the first time in Turkey. According to this policy our aim; for all persons of the EU communities, including university all members have the right to pursue their goals in a healthy and safe university, with effective and legal drug monitoring system. Within the framework of this policy, we aim to organize an effective drug monitoring system within collaboration of protective and educative approaches especially among young people and whole campus personnel. By this way we’ll able to get real concrete database.

Methods
In order to prevent the use of alcohol and illicit substances in the campus and working places, “Drug Free Campus and Workplace Policy” is approved by the EU. This policy is implemented in compliance with the Turkish Penal Code, Human Rights, and Board of Higher Education Students (BHES) to promote the mission of the university. The Medical Laboratory Regulations has been registered by the Ministry of Health in Turkey; illegal substance analysis is based on a two-step analysis strategy. With this strategy, authorized labs are responsible to carry out immunochemical screening analyzes for urine samples and confirmation analyzes in urine samples with chromatographic methods using gas chromatography mass spectrometer (GC-MS). Turkish guideline includes these substances and cut-off values: amphetamines-500 ng/mL, benzodiazepines-300 ng/mL, cannabis(THC-COOH)-50 ng/mL, cocaine-150 ng/mL, opiates-2000 ng/mL are analyzed by immunochemical methods. Our institute's screening and confirmation analyses cut-off levels are compatible with this panel.

Results
Main steps of this policy are publicity, education, care/treatment, drug monitoring and sanctions.

Publicity part: University of public relations department and communication faculty generating publicity by utilizing the media.

Educational part: BATI Institute and associated faculties; description of the risks related with the use of illicit drugs and the abuse of alcohol, theoretical and trainings, awareness activities.

Care/Treatment part: BATI Institute and associated faculties provide a reasonable level of care for substance abusers through counseling, treatment, and rehabilitation.

Drug Monitoring Part: Screening and confirmation is applied to clarify University person’s drug and alcohol using situation and get the analytical data, for treatment and forensic situations. When circumstances suggest impairment, reasonable suspicion etc., EU member will be asked to submit to drug screen test or blood alcohol test. The Laboratory will take possession of the Chain of Custody/Test Requisition form and initiate all activities associated with the drug testing program in accordance with their departmental policy and according to international guidelines. It should be realized in this policy scope, drug monitoring part is providing evidence. For protecting any deficiency and providing justice, homogeneous and reliable regulations of drug testing systems should standardized, for this reason our Institute has a main responsibility.

Sanctions part: The disciplinary actions based on the legislations.

Discussion
In 1989 Drug Free Schools and Communities Act are carried out activities aiming at universities away from the harmful effects of alcohol. Since the 1990s, the US, the UK, Canada, and China have started work on the creation of services and programs for young people on substance and alcohol use. Some of these above mentioned universities; to reduce substance use have been working with the Federal Government. One of these programme is “Screening, Brief Intervention, Referral and Treatment” system in US. Our policy is also related government departments. Universities are important to guide the future “Drug Free Campus and Workplace Policy” is designed by BATI Institute for the first time in Turkey. Turkish Penal Code and BHES legislations are compatible with international Drug Free Campus amendments in our country. But the lack of specific legislations on fighting illicit substances for university campuses is a concern and the drug monitoring and sanctions parts regulations are not complemented yet.

Conclusions
BATI Institute founded to keep struggle with drug abuse and addiction. To constitute drug free campus, spread consciousness of multidimensional approach to our society, drug monitoring part is very important for deterrence to drug use for treatment/supervising and as forensic evidence. According to this situation, as the BATI Institute will continue to combat illegal substances as the locomotive of this policy in the detection and monitoring of illegal substance presence on campus by conducting drug tests as part of the campus policy. So far there is no request for drug testing to our Institute according with this policy.