ABSTRACT BOOKLET

61ST

ANNUAL MEETING

TIMFI

2024

ST.GALLEN

SEPTEMBER 2ND – 6TH

OLMA MESSEN

DEAR COLLEAGUES AND FRIENDS

On behalf of the Organizing Committee, it is our great pleasure to welcome you to the TIAFT 2024 Conference in the beautiful city of St.Gallen, Switzerland. This year, we gather once again as a global community of experts in forensic toxicology to share our latest research, innovations, and developments in the field.

St.Gallen, renowned for its stunning landscapes and rich cultural heritage, provides a perfect backdrop for this conference. It is a city that embodies the blend of tradition and innovation, much like TIAFT itself. We hope you will take the opportunity to explore its charming streets, historical landmarks, and enjoy the warm hospitality of its people.

We have put in all our efforts to ensure that the TIAFT 2024 conference will be an engaging and intellectually stimulating event. Our program includes a diverse range of presentations and vendor workshops, but we will also encourage social exchange. For the regular attendees of TIAFT conferences, you know that the events are like an annual gathering of a family. All newbies will be accepted into this global family of forensic toxicology.

This abstract book is a testament to the hard work and dedication of our contributors. It features a collection of cutting-edge research and case studies that reflect the dynamic and evolving nature of our discipline. We are confident that these abstracts will inspire fruitful discussions and collaborations throughout the conference.

We extend our heartfelt gratitude to all the authors, speakers, and participants for their invaluable contributions. Special thanks are also due to our sponsors and partners for their generous support, without which this event would not be possible.

Thank you for joining us at TIAFT 2024. We wish you a memorable and rewarding conference experience in St.Gallen.

Warmest regards,

Dr. Jochen Beyer
Chair of the TIAFT 2024 Organizing Committee

WELCOME

Dear colleagues

Welcome to the 61st Annual Meeting of TIAFT in St.Gallen.
I would like to extend my gratitude to the organisers of this meeting, especially Jochen Beyer. For me it is quite symbolic that my last year as TIAFT president will conclude here in Switzerland at this annual meeting. For those of you who are unaware of our history together, we first met in 2004 at the Washington DC SOFT/TIAFT meeting. A few years later, Jochen travelled to Australia to assist us with developing LC/MS techniques for our coronial casework. Jochen eventually moved to Switzerland managing the toxicology laboratory at the Institute of Forensic Medicine in St.Gallen. Fast forward to 2024, and it is hard to believe that TIAFT members (including me) would be hosted by my good friend!
It is my pleasure to welcome you all to this conference and over the coming days you will hear presentations that highlight new research, innovative methodologies and developments in our discipline that contribute to promoting and better understanding forensic toxicology. I would also like to thank all our presenters, conference organisers, sponsors and partners who make such large scientific meetings possible, and also memorable. As I have suggested in many of my presidents' messages to the membership, please make some time to meet someone new at this meeting – you never know what opportunities may arise from scientific collaborations that end up being very rewarding, both from a professional and personal perspective. We welcome our colleagues from SOHT (many of us are already members of this association) and look forward to inspiring and fruitful scientific discussions.

Dimitri Gerostamoulos
TIAFT President

Dear colleagues

Welcome to the 61st Annual Meeting of TIAFT in St. Gallen.
I would like to welcome you to this joint meeting between the Society of Hair Testing and The International Association of Forensic Toxicologists here in St.Gallen. To those members present, I would like to extend a special invitation to the SoHT General Assembly which takes place on the first day of the meeting, Monday September 2nd. On the Wednesday, there will be two scientific sessions dedicated to the analysis and interpretation of hair. However, joint meetings are important because one can engage also with researchers, forensic and clinical professionals from many different fields and benefit from scientific talks outside one's own expertise. So, please take the opportunity to meet new people. I am looking forward to seeing many old and new faces in St.Gallen.

Robert Kronstrand
SoHT President

The city of St.Gallen

COMMITTEES

Local Organizing and Scientific Committee

Jochen Beyer – Chair
Björn Moosmann – Scientific
Annette Cronin – Scientific
Andrea Steuer – Scientific
Sascha Steinmann – Olma Congress
Nadine Bleiker – Olma Congress
Rahel Candrian – Communication and Design
Ladina Büttiker – Administration
Markus Baumgartner – Public Relations

International Scientific Committees

TIAFT

Serap Annette Akgür, Turkey
Hilke Andresen-Streichert, Germany
Volker Auwärter, Germany
Federica Bortolotti, Italy
Marta Concheiro-Guisan, USA
Bronwen Beth Davies, South Africa
Brigitte Desharnais, Canada
Teemu Gunnar, Finnland
Islam-Amine Larabi, France
Luca Morini, Italy
Frank Theodor Peters, Germany
Luke Rodda, USA
Jennifer Lucinda Schumann, Australia
Peter Stockham, Australia
Helena Teixeira, Portugal
Shimpei Watanabe, Japan
Dariusz Zuba, Poland

SoHT

Maristela Andraus, United Kingdom
Brice Appenzeller, Luxembourg
Tina Binz, Switzerland
Vincent Cirimele, France
Gail Cooper, USA/Scotland
Maria del Mar Ramirez Fernandez, Belgium
Donata Favretto, Italy
Robert Kronstrand, Sweden
Frank Sporkert, Switzerland
Sabina Strano Rossi, Italy
Michel Yegles, Luxembourg

International Advisory Panel

TIAFT EXECUTIVE COMMITTEE

President: Dimitri Gerostamoulos, Australia
President Elect: Simon Elliott, United Kingdom
Past President: Marc LeBeau, USA
Secretary: Carmen Jurado, Spain
Treasurer: Christophe Stove, Belgium
Board Members: Jean Claude Alvarez, France; Nicolas Lemos, USA; Sarah Wille, Belgium

SoHT EXECUTIVE COMMITTEE

President: Robert Kronstrand, Sweden
Vice President: Brice M.R. Appenzeller, Luxembourg
Secretary: Maristela Andraus, United Kingdom
Treasurer: Frank Sporkert, Switzerland
Board Members: Mario Barrosso, Portugal; Tina Binz, Switzerland; Vincent Cirimele, France; Donata Favretto, Italy; Maria del Mar Ramirez, Belgium; Sabina Strano Rossi, Italy; Michel Yegles, Luxembourg

YOUNG SCIENTISTS MEETING

Monday, 2nd September 2024
09:00 – 12:30
Lecture hall (9.1.B)

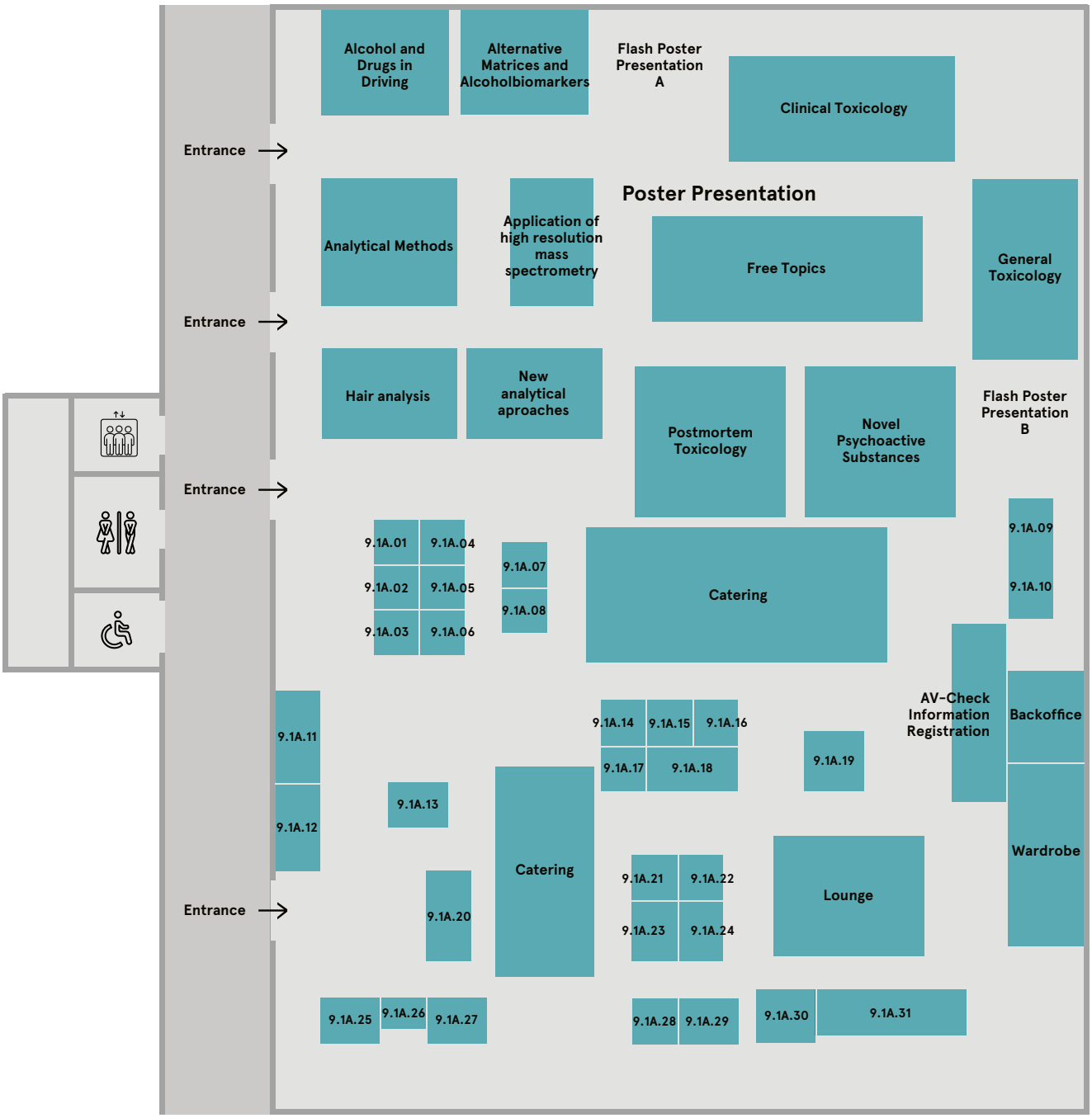
YOUNG SCIENTISTS COMMITTEE

Chair: Luke Rodda, USA
Secretary: Elena Lendoiro, Spain

Committee Members
Francesco Busardò, Italy
Bronwen Davies, South Africa
Sarah Eller, Brazil
Daniel Pasin, Australia
Lea Wagmann, Germany
Shimpei Watanabe, Japan

09:00 – 09:10	**Welcome remarks Young Scientists Committee**
09:10 – 09:15	**Welcome remarks from St.Gallen Host** – Jochen Beyer, Switzerland
09:15 – 09:45	**YS Welcome and Introductions**
09:45 – 10:15	**YS Award 2023 (Poster)** – Laura Franke, Germany Urine Adulteration by Synthetic Urine: Indirect and Direct Detection Approaches
10:15 – 10:45	**YS Award 2023 (Oral)** – Andre Luis Fabris, Brazil Combining drug analysis with green chemistry
10:45 – 11:00	Short Break
11:00 – 11:15	**YSC Mentor Talk** – Luke Rodda, USA Navigating challenges and growth during the early to mid-career journey
11:15 – 11:50	**Mentor talk** – Suman Rana, USA Navigating the path to success through mentorship, resilience and vulnerability
11:50 – 12:30	**Mentor talk** – Markus Baumgartner, Switzerland Keratinised matrices: Pitfalls and prospects

EXHIBITION AREA



9.1A.02 Restek Corporation
9.1A.03 Siemens Healthineers AG
9.1A.04 Merck Life Science KGaA
9.1A.05 Chiron AS
9.1A.06 Drägerwerk AG
9.1A.09 TIAFT 2026
9.1A.10 TIAFT 2025
9.1A.11 Kantonspolizei St.Gallen
9.1A.12 Breath Explor Munkplast AB
9.1A.13 Chromsystems GmbH
9.1A.14 ARK Diagnostics Inc.
9.1A.15 United ChemicalsTechnologies Inc.
9.1A.16 Securetec Detektions-System AG
9.1A.17 LGC
9.1A.18 Thermo Fisher Scientific
9.1A.19 Drug Checking
9.1A.20 Shimadzu
9.1A.21 Instrumentation Laboratory SpA – A Werfen Company
9.1A.22 ACQ Science
9.1A.23 Biotage Sweden AB
9.1A.24 Bruker Daltonics GmbH & Co.KG
9.1A.25 Waters AG
9.1A.26 MEDICHEM Diagnostica GmbH
9.1A.27 Randox Toxicology
9.1A.28 EUREKA S.R.L. LAB DIVISION
9.1A.29 AB SCIEX Switzerland GmbH
9.1A.30 Barista «cofftales» by Dorner GmbH
9.1A.31 Souvenir-Shop

PROGRAM OVERVIEW

MONDAY, 2ND SEPTEMBER 2024

08:00 – 11:30	Board Meeting TIAFT	9.0 C
08:00 – 11:30	Board Meeting SoHT	9.0 D
09:00 – 12:30	Young Scientists Meeting TIAFT	Lecture hall (9.1.B)
12:30 – 13:30	Break (Lunch for Young Scientists and Board members only)	
13:30 – 16:00	SoHT Annual General Meeting	Lecture hall (9.1.B)
13:30 – 14:30	Regional Representative Meeting TIAFT	9.0 A
16:00 – 17:30	Vendor Workshop – Thermo Fisher Scientific	9.0 B
17:30 – 19:00	Opening Ceremony	Lecture hall (9.1.B)
19:00 – 21:00	Welcome Reception and Exhibition opening	Exhibition hall (9.1.A)
21:00	TIAFT Pub crawl	St.Gallen Downtown

TUESDAY, 3RD SEPTEMBER 2024

07:30 – 08:30	Vendor Workshop – Shimadzu	9.0 A
07:30 – 08:30	Vendor Workshop – Sciex	9.0 B
08:30 – 10:00	Scientific Session 1 – Applications of high resolution mass spectrometry	Lecture hall (9.1.B)
10:00 – 10:30	Break and Exhibition	Exhibition hall (9.1.A)
10:00 – 10:30	Poster gallery – NPS F-P-1 to P-51	Exhibition hall (9.1.A)
10:30 – 12:30	Scientific Session 2 – Novel psychoactive substances I	Lecture hall (9.1.B)
12:30 – 14:00	Break and Exhibition	Exhibition hall (9.1.A)
13:15 – 13:45	Flash Poster Presentation – NPS I	9.1.A – Flash Poster Area A
13:15 – 13:45	Flash Poster Presentation – SOHT	9.1.A – Flash Poster Area B
14:00 – 16:00	Scientific Session 3 – New analytical approaches	Lecture hall (9.1.B)
16:00 – 16:30	Break and Exhibition	Exhibition hall (9.1.A)
16:00 – 16:30	Poster gallery – AM F-P-1 to P-43	Exhibition hall (9.1.A)
16:00 – 16:30	Poster gallery – NAA P-1 to P-18	Exhibition hall (9.1.A)
16:30 – 18:00	Scientific Session 4 – Alternative matrices and alcohol biomarkers	Lecture hall (9.1.B)
21:00 – 23:59	Young Scientists Night Out	St.Gallen – BrüW pub

WEDNESDAY, 4TH SEPTEMBER 2024

06:30 – 07:30	TIAFT Fun Run	Bus stop Rotmonten
08:30 – 10:00	Scientific Session 5 – Hair analysis I, joint with SOHT	Lecture hall (9.1.B)
10:00 – 10:30	Break and Exhibition	Exhibition hall (9.1.A)
10:00 – 10:30	Poster gallery – SOHT F-P-1 to P-25	Exhibition hall (9.1.A)
10:00 – 10:30	Poster gallery – CT F-P-1 to P-26	Exhibition hall (9.1.A)
10:30 – 12:30	Scientific Session 6 – Hair analysis II, joint with SOHT	Lecture hall (9.1.B)
12:30 – 13:00	Break	Exhibition hall (9.1.A)
13:00 – 23:00	Excursion, booked separately, at surcharge	In front of hall 9

THURSDAY, 5TH SEPTEMBER 2024

07:30 – 08:30	Vendor Workshop – Waters	9.0 B
08:30 – 10:00	Scientific Session 7 – Drugs in driving and on-site testing	Lecture hall (9.1.B)
10:00 – 10:30	Break and Exhibition	Exhibition hall (9.1.A)
10:00 – 10:30	Poster gallery – DUI F-P-1 to P-17	Exhibition hall (9.1.A)
10:00 – 10:30	Poster gallery – GT P-1 to P-29	Exhibition hall (9.1.A)
10:30 – 12:30	Scientific Session 8 – Alcohol and drugs and driving	Lecture hall (9.1.B)
12:30 – 14:00	Break and Exhibition	Exhibition hall (9.1.A)
13:15 – 13:45	Flash Poster Presentation – Postmortem toxicology	9.1.A – Flash Poster Area A
13:15 – 13:45	Flash Poster Presentation – Free topics	9.1.A – Flash Poster Area B
14:00 – 16:00	Scientific Session 9 – Clinical toxicology	Lecture hall (9.1.B)
16:00 – 16:30	Break and Exhibition	Exhibition hall (9.1.A)
16:00 – 16:30	Poster gallery – HR F-P-1 to P-11	Exhibition hall (9.1.A)
16:00 – 16:30	Poster gallery – DIV F-P-1 to P-32	Exhibition hall (9.1.A)
16:30 – 18:00	Scientific Session 10 – Analytical methods	Lecture hall (9.1.B)
18:00 – 22:00	Vendor Workshop – Biotage	9.0 B

FRIDAY, 6TH SEPTEMBER 2024

08:30 – 10:00	Scientific Session 11 – Postmortem toxicology	Lecture hall (9.1.B)
10:00 – 10:30	Break and Exhibition	Exhibition hall (9.1.A)
10:00 – 10:30	Poster gallery – PM F-P-1 to P-48	Exhibition hall (9.1.A)
10:00 – 10:30	Poster gallery – AB P-1 to P-12	Exhibition hall (9.1.A)
10:30 – 12:30	Scientific Session 12 – Novel psychoactive substances II	Lecture hall (9.1.B)
12:30 – 14:00	Break and Exhibition	Exhibition hall (9.1.A)
13:15 – 13:45	Flash Poster Presentation – NPS II	9.1.A – Flash Poster Area A
13:15 – 13:45	Flash Poster Presentation – Free topics II	9.1.A – Flash Poster Area B
14:00 – 15:00	Scientific Session 13 – Free topics	Lecture hall (9.1.B)
15:00 – 15:15	Short Break	Exhibition hall (9.1.A)
15:15 – 18:00	TIAFT Annual General Meeting	Lecture hall (9.1.B)
19:30	Conference Dinner and Awards Ceremony	Hall 2

SCIENTIFIC PROGRAM

- Ceremony & Events
- Scientific Session
- Flash Poster Presentation
- Poster gallery
- Break and Exhibition
- Vendor Workshop

MONDAY, 2ND SEPTEMBER 2024

08:00 – 09:00	Board Meeting TIAFT	
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17:30 – 19:00	Opening Ceremony	
19:00 – 21:00	Welcome Reception and Exhibition opening	
21:00	TIAFT Pub crawl	

TUESDAY, 3RD SEPTEMBER 2024

07:30 – 08:30	Vendor Workshop – Shimadzu	Vendor Workshop – Sciex
08:30 – 10:00	**Scientific Session 1 – Applications of high resolution mass spectrometry** Chair: Ilkka Ojanperä, Daniel Pasin 08:30 – 08:42 [HR O-1] **Feasibility of using routine forensic toxicology data for metabolomics studies exemplified for amphetamine** Annina Bovens, Claudio Leu, Thomas Kraemer, Andrea E. Steuer 08:42 – 08:54 [HR O-2] **Characterizing consumption markers of the designer benzodiazepine bretazenil by high-resolution mass spectrometry using human hepatocytes and authentic postmortem samples** Prince S. Gameli, Johannes Kutzler, Laura M. Huppertz, Francesco Paolo Busardò, Jeremy Carlier, Volker Auwärter 08:54 – 09:06 [HR O-3] **Development and optimization of compound identification scoring and data automation in high-resolution mass spectrometry analysis** Maria Sarkisian, Megan C Farley, Luke N Rodda 09:06 – 09:18 [HR O-4] **Postmortem metabolomics and the application of machine-learning to predict postmortem interval** Liam J. Ward, Jenny Arpe, Carl Söderberg, Albert Elmsjö, Rasmus Magnusson, William Lövfors, Fredrik C. Kugelberg, Elin Nyman, Henrik Gréen 09:18 – 09:30 [HR O-5] **Data analysis workflows utilizing SQL for UHPLC-orbitrap and ion trap MS data in untargeted metabolomics** Pia Johansson Heinsvig, Christian Brinch Mollerup, Marie Mardal 09:30 – 09:42 [HR O-6] **Drug concentration prediction in forensic toxicology utilizing high-resolution QTOF tox screening** Albert Elmsjö, Liam J. Ward, Carl Söderberg, Fredrik C. Kugelberg, Henrik Gréen 09:42 – 09:54 [HR O-7] **A metabolomics approach for CYP phenotyping: A proof-of-concept to use enzyme-based study designs** Yannick Wartmann, Thomas Kraemer, Andrea E. Steuer	
10:00 – 10:30	Break and Exhibition	Poster gallery – NPS F-P-1 to P-51

10:30 – 12:30

Scientific Session 2 – Novel psychoactive substances I

Chair: Matthew Hosking, Carmen Jurado

10:30 – 10:43 [NPS O-1]
Update from the EU Early Warning System on new psychoactive substances
Joanna De Morais, Michael Evans-Brown, Rachel Christie, Rita Jorge, Gregorio Planchuelo, Katarzyna Berkowicz, Thomas Nefau, Ana Gallegos, Roumen Sedefov

10:43 – 10:56 [NPS O-2]
Current state and recent advances of HighResNPS for suspect screening of new psychoactive substances
Daniel Pasin, Peter Stockham

10:56 – 11:09 [NPS O-3]
Changing trends in synthetic cannabinoid receptor agonist (SCRA) use in Scottish prisons: detection, prevalence and modes of use
Victoria Marland, Caitlyn Norman, Robert Reid, Craig McKenzie, Herve Menard, Anne Coxon, Niamh Nic Daeid

11:09 – 11:22 [NPS O-4]
Epidemiology of new psychoactive substances in relation to traditional drugs of abuse in clinical oral fluid samples collected in Sweden
Magnus AB Axelsson, Hanna Lövgren, Robert Kronstrand, Henrik Gréen, Moa Andresen Bergström

11:22 – 11:35 [NPS O-5]
Harm caused by metonitazene in New Zealand during 2022 and 2023
Diana Kappatos, Olivia Johnson, Sarah Russell, Jessica Baker, Lucy Stiles, Mathew Hosking

11:35 – 11:48 [NPS O-6]
Chemical analysis of recreational products containing semi-synthetic cannabinoids in Germany
Benedikt Pulver, Benjamin Schmitz, Simon D. Brandt, Volker Auwärter

11:48 – 12:01 [NPS O-7]
Hemp-derived or semisynthetic cannabinoids: pharmacology of isomers and how it matters for the harm potential of seized drugs
Liesl K. Janssens, Katleen Van Uytfanghe, Jeffrey Williams, Kirk W. Hering, Donna M. Iula, Christophe P. Stove

12:01 – 12:14 [NPS O-8]
Isomeric analysis of hexahydrocannabinol (HHC), hexahydrocannabihexol (HHCH), and hexahydrocannabiphorol (HHCP) in products using LC-MS and supercritical fluid chromatograph (SFC)-QTOF-MS
Ruri Kikura-Hanajiri, Maiko Kawamura, Sakumi Mizutani, Rie Tanaka, Genichiro Tsuji, Takashi Misawa, Hidetomo Yokoo, Yosuke Demizu

12:14 – 12:27 [NPS O-9]
Another new addition to the SCRA landscape: in vitro characterization of the functional activity and metabolites of CHO-4'Me-5'Br-FUBOXPYRA
Marie H. Deventer, Brianna N. Stang, Claude Guillou, Alex J. Krotulski, Christophe P. Stove

12:30 – 13:15 Break and Exhibition

13:15 – 13:45

Flash Poster Presentation – NPS I

Chair: Marthe Vandeputte

13:15 – 13:20 [NPS F-P-01]
A comprehensive HPLC-MS/MS method for the detection of hexahydrocannabinol (HHC) epimers and related metabolic profile and its application to in vitro metabolic studies of stereochemically pure and mixture of HHC epimers
Milena Pajovic, Kim Wolff, Erika Castrignanò

13:20 – 13:25 [NPS F-P-02]
Metabolic biotransformation of synthetic cannabinoid receptor agonists (SCRAs)
Eathan Walker, Eric Sparkes, Morgan Alonzo, Unnikrishnan Kuzhiumparambil, Shanlin Fu

13:25 – 13:30 [NPS F-P-03]
Analysis of over 250 novel synthetic opioids and xylazine by LC–MS-MS in blood and urine
Katie Diekhans, Jihau Yu, Megan C Farley, Luke N Rodda

Flash Poster Presentation – SOHT

Chair: Maria del Mar Ramirez Fernandez

13:15 – 13:20 [SOHT F-P-01]
Revealing polydrug abuse in ketamine users: Insight from hair analysis on drug consumption patterns
Hooi Yan Moy, HuiFen Hannah Zhang, Si Jia Chan, Chi Pang Lui

13:20 – 13:25 [SOHT F-P-02]
The detection of AEME in hair samples where thermal hair straighteners have been used
Donna M Muldoon, Angharad John, Charlotte Peake

13:25 – 13:30 [SOHT F-P-03]
Forensic challenges: Distinguishing methamphetamine consumption from external contamination
Maggie Su Su Tiong, Si Jia Chan, Huifen Zhang, Ngak Lee Tan-Lee, Hooi Yan Moy, Chi Pang Lui

13:15 – 13:45	Break and Exhibition	13:30 – 13:35 [NPS F-P-04] **Deaths involving novel benzodiazepines in Victoria, Australia** Olaf H. Drummer, Samantha Joubert, Matthew Di Rago, Jared W. Castle, Kerryn Crump, Linda Glowacki, Dimitri Gerostamoulos 13:35 – 13:40 [NPS F-P-05] **HHC and derivatives – method development for analysis of semi-synthetic cannabinoids and their metabolites and its application to routine samples** Marica Hundertmark, Laura Besch, Jörg Röhrich, Cora Wunder	13:30 – 13:35 [SOHT F-P-04] **Application of hair analysis in pediatric post-mortem toxicology** Ayako Chan-Hosokawa, Stephanie M Marco, Lorenzo Gitto 13:35 – 13:40 [SOHT F-P-05] **Cerumen as an alternate bio-matrix for drugs of abuse: correlation of analysis results with those for urine, blood and hair** Zeerak Abbas, Nadeem Ul Hassan Khan, Muneeb Arshad
13:45 – 14:00			
14:00 – 16:00	**Scientific Session 3 – New analytical approaches** Chair: Andrea Steuer, Christophe Stove 14:00 – 14:13 [NAA O-1] **Development of a multi-omics sample preparation workflow for comprehensive Metabolomics, Lipidomics and Proteomics datasets using a single tissue sample** Lana Brockbals, Maiken Ueland, Shanlin Fu, Matthew P. Padula 14:13 – 14:26 [NAA O-2] **Acoustic ejection tandem mass spectrometry for high-throughput screening of phencyclidine-type substances in urine, including authentic cases** Yan Shi, Ziyi Li, Wei Liu, Ping Xiang 14:26 – 14:39 [AM O-1] **A new alternative for dried blood spots papers: nanofibrous sorbents** Melike Aydoğdu, Tuğçe Töngüç Yalçınkaya, Ahmet Çay, Emriye Perrin Akçakoca, Fatma Nil Ertaş, Serap Annette Akgür 14:39 – 14:52 [NAA O-4] **Comprehensive characterization of biological fluids from subjects tested positive for illicit drugs using supercritical fluid chromatography mass spectrometry with orthogonal ionization and fragmentation techniques** Patrick Mueller, Stefan König, Gérard Hopfgartner 14:52 – 15:05 [NAA O-5] **Drug characterization and impurity mapping using R** Alasoul Saif, Herve Menard, Lorna Nisbet 15:05 – 15:18 [NAA O-6] **Development of a green analytical toxicology method based on supramolecular solvent (SUPRAS) microextraction for the analysis of benzodiazepines in vitreous humor** Kauê O. Chinaglia, Leonardo C. Rodrigues, Lohanna P. El Haddad, Laura S. Borbi, Débora Z. Berlinck, José L. Costa 15:18 – 15:31 [NAA O-7] **Activity-based characterization of opiates, opioids and NPS molecules, including their metabolites: a forgotten technology with new forensic applications** Jan Karel P. Tytgat 15:31 – 15:44 [NAA O-8] **Application of chemometrics in the interpretation of diclazepam and its metabolite ratios** Christopher Davies, Agatha Grela, Joanne Morrissey, Havovi Chichger, Philip Pugh, Lata Gautam 15:44 – 15:57 [NAA O-9] **Exploring electromembrane extraction as a simple and green alternative for the analysis of New Psychoactive Substances** André Fabris, Frederik André Hansen, Mauricio Yonamine, Stig Pedersen-Bjergaard, Elisabeth Leere Øiestad	
16:00 – 16:30	Break and Exhibition	Poster gallery – AM F-P-1 to P-43	Poster gallery – NAA P-1 to P-18
16:30 – 18:00	**Scientific Session 4 – Alternative matrices and alcohol biomarkers** Chair: Sarah Wille, Yi Ju Yao 16:30 – 16:42 [AB O-1] **Comparison of PEth in blood and ethyl glucuronide in hair in Driving Aptitude Assessment** Matthias Bantle, Matthias Pfäffli, Wolfgang Weinmann 16:42 – 16:54 [AB O-2] **Automated sample preparation as leverage for high-throughput drug screening of wastewater** Marie Mardal, Kristian Linnet, Marie Katrine Klose Nielsen, Sys Stybe Johansen, Brian Schou Rasmussen, Ragnar Thomsen		

16:30 – 18:00	16:54 – 17:06 [AB O-3] LC-MS/MS method for monitoring antiepileptic drugs in DBS. Comparison of DBS, VAMS and whole blood results. María Cobo-Golpe, Lucía Paniagua-González, Miriam Blanco-Ces, Angelines Cruz, Elena Lendoiro, Ana de-Castro-Ríos 17:06 – 17:18 [AB O-4] Informative value of phosphatidylethanol (PEth) in post-mortem blood samples Jeremai Hose, Martin Juebner, Mario Thevis, Hilke Andresen-Streichert 17:18 – 17:30 [AB O-5] Could ethylated phosphorylcholine be a new marker for ethanol consumption? Catalina Dumitrascu, Elias Iturrospe, Celine Gys, Matthias Van Puymbroeck, Glenn Van Nieuwenhove, Maryline Busschots, Diona D'Hondt, Alexia Van Goethem, Wout Claeys, Babette Van Rafelghem, Eline Baetens, Werner Jacobs, Hugo Neels, Adrian Covaci, Alexander L.N. van Nuijs 17:30 – 17:42 [AB O-6] Prevalence of substance use identified in oral fluid and hair within a large-scale US court-ordered mandatory drug testing population Jeri D. Ropero-Miller, Megan Grabenauer, Katherine M. Bollinger, Nicolas J. Richardson, David C. Heller 17:42 – 17:54 [AB O-7] Dental calculus as alternative matrix – evaluation of drug findings in eight postmortem cases Merja A. Neukamm, Kerstin Henkel, Volker Auwärter, Markus J. Altenburger
21:00 – 23:59	Young Scientists Night Out

WEDNESDAY, 4TH SEPTEMBER 2024

06:30 – 07:30	Fun Run/Walk
08:30 – 10:00	Scientific Session 5 – Hair analysis I, joint with SOHT Chair Tina Binz, Alberto Salomone 08:30 – 08:42 [SOHT O-1] Cortisol in hair as a stress biomarker in pregnant women and its correlation with demographics and clinical outcomes Rachel Calvagna, Melissa Orr, Mamta Fuloria, Maureen J Charron, Marta Concheiro 08:42 – 08:54 [SOHT O-2] Advancements in forensic hair analysis using UPLC- Q-TOF and UPLC-MS/MS demonstrated via a case report concerning synthetic cathinone abuse Maria del Mar Ramirez Fernandez, Sarah M.R. Wille, Vincent Di Fazio, Joy Eliaerts, Nele Samyn 08:54 – 09:06 [SOHT O-3] Hair analysis as an epidemiological indicator to assess the drug situation in Europe Christina Ververi, João Matias, Pamela Dugues, Jean-Claude Alvarez, Daniel Martins, Olesia Rudminienė, Enrico Gerace, Marta Massano, Alberto Salomone, Islam Amine Larabi 09:06 – 09:18 [SOHT O-4] Chiral analysis of ketamine in hair by UHPLC-MS/MS Xin Wang, Zhen Zhang, Ping Xiang 09:18 – 09:30 [SOHT O-5] Using a hair reference material as a benchmark to evaluate drug testing performance in five US hair testing laboratories and work towards harmonization and consensus. Svante Vikingsson, E Dale Hart, Ruth E Winecker, Eugene D Hayes, Ronald R Flegel, Lisa S. Davis 09:30 – 09:42 [SOHT O-6] Evaluation of the influence of keratin hair straightening on the detection of xenobiotics, illustrated with caffeine and endogenous GHB- preliminary study. Karolina Nowak, Paweł Szpot, Marcin Zawadzki 09:42 – 09:54 [SOHT O-7] Analyte recoveries from intact and powdered authentic hair at different extraction conditions. Robert Kronstrand, Markus Roman, Gerd Jakobsson

10:00 – 10:30	Break and Exhibition	Poster gallery – SOHT F-P-1 to P-25	Poster gallery – CT F-P-1 to P-26
10:30 – 12:30	**Scientific Session 6 – Hair analysis II, joint with SOHT** Chair: Marc LeBeau, Frank Sporkert 10:30 – 10:43 [SOHT O-8] **Detection of pyrazolam in hair by LC-MS/MS in two drug-facilitated-crime cases** Gerd Jakobsson, Robert Kronstrand 10:43 – 10:56 [SOHT O-9] **Unanticipated results of hair analysis for synthetic cannabinoids linked to worn headwear: A case report** Annette Zschiesche, Yvonne S. Jungel, Laura M. Huppertz, Merja A. Neukamm, Volker Auwärter 10:56 – 11:09 [SOHT O-10] **Hair analysis after single and repeated short in vivo passive exposures to cannabis and "cannabis light": preliminary results** Arianna Giorgetti, Guido Pelletti, Simone Santelli, Susan Mohamed, Francesca Rossi, Jennifer P Pascali 11:09 – 11:22 [SOHT O-11] **The stability of Ethyl Glucuronide (EtG) in stored hair samples** Amy Duda, Lolita Tsanaclis 11:22 – 11:35 [SOHT O-12] **Development and validation of an HPLC-MS/MS method for the determination of benzodiazepines in hair. Comparison of two different extractions and applications on real samples.** Giulia Stocchero, Martina Pes, Manuela Lucchiari, Antonio Cambria, Giovanni Michele Marchio, Flora Donini, Francesco Ingenito, Lucia Bertoldi, Cristiana Fait, Lucia Pecoraro, Adriano Anesi, Alberto Salomone, Donata Favretto 11:35 – 11:48 [SOHT O-13] **Endocannabinoid and steroid levels during and after pregnancy in fingernails of mothers and their newborns** Clarissa D. Vögel, Tanja Restin, Pearl La Marca-Gaemmaghami, Matilde Murari, Thomas Kraemer, Tina M. Binz 11:48 – 12:01 [SOHT O-14] **Hair analysis of critically ill pediatric patients undergoing opioid treatment with focus on fentanyl derivatives and metabolites** Max C. Polke, Florian Zapf, Tanja Restin, Thomas Kraemer, Tina M. Binz 12:01 – 12:14 [SOHT O-15] **Retrospective evaluation of novel synthetic opioids and xylazine chronic intake by post-mortem hair analysis** Rossella Gottardo, Marco Ballotari, Nicola Pigaiani, Karen S Scott, Gregory G Davis, Federica Bortolotti 12:14 – 12:27 [SOHT O-16] **Hydroxyketamine and hydroxynorketamine isomers can be detected in hair of ketamine users** Giampietro Frison, Flavio Zancanaro, Alessia Finotello, Samuela Frasson, Silvano Zancaner		
12:30 – 13:00	Break		
13:00 – 23:00	Excursion, booked separately, at surcharge		

THURSDAY, 5TH SEPTEMBER 2024

07:30 – 08:30	Vendor Workshop – Waters		
08:30 – 10:00	**Scientific Session 7 – Drugs in driving and on-site testing** Chair : Marilyn Huestis, Nikolas Lemos 08:30 – 08:42 [DUI O-1] **Validation of an LC-MS/MS method for screening 340 classical Drugs of Abuse (cDoA), NPS, and pharmaceuticals: Application to the analysis of residues from used syringes within the ESCAPE network** Islam Amine Larabi, Pamela Dugues, Emuri Abe, Gemara Beaufils, Catherine Duplessy, Thomas Nefau, Thomas Seyler, Jean-Claude Alvarez 08:42 – 08:54 [DUI O-2] **Preliminary pharmacokinetic and psychophysical data after controlled inhalative and oral consumption of hexahydrocannabinol (HHC)** Lisa Höfert, Benjamin Franz, Cedric Groß, Jan Dreßler, Susen Becker, Sven Baumann 08:54 – 09:06 [DUI O-3] **The development of a D-9-THC colorimetric breathalyzer using 3D printing manufacture** Savanah Allinson, Aron Jaffe, Taqiyah Chernesky, Stephanie Walcott, Emanuele A Alves 09:06 – 09:18 [DUI O-4] **On-site instrumental analysis in a toxicology context: towards portable capillary electrophoresis for determining gamma-hydroxybutyric acid (GHB) at the point of need** Giacomo Musile, Marc-Aurèle Boillat, Goetz Schlotterbeck, Peter Hauser 09:18 – 09:30 [DUI O-5] **Development, validation, and application of a high-throughput automated sample preparation technique** Tyler Devincenzi, Steven R Towler, Luke N Rodda 09:30 – 09:42 [DUI O-6] **Development of a paper-based presumptive test for the detection of fentanyl and its analogues** Archie Lambrinos, Morgan Alonzo, Shanlin Fu 09:42 – 09:54 [DUI O-7] **Prevalence and blood concentration of drugs in fatal road accidents in Western Switzerland from 2002 to 2023** Marc Augsburger, Jonathan Maurer, Nicolas Donzé, Claudia Castiglioni, Tony Fracasso, Aurélien Thomas		
10:00 – 10:30	Break and Exhibition	Poster gallery – DUI F-P-1 to P-17	Poster gallery – GT P-1 to P-29
10:30 – 12:30	**Scientific Session 8 – Alcohol and drugs and driving** Chair: Brice Appenzeller, Marc Augsburger 10:30 – 10:43 [DUI O-8] **Prevalence of alcohol, drugs of abuse and medicines in fatal road traffic injuries in a Thai population between 2018 and 2023** Patcharanun Chulamanee, Peerayuht Phuangphung 10:43 – 10:56 [DUI O-9] **Drugs of abuse detected in oral fluid samples of Victorian drivers over 15 years (2008-2022).** Matthew Di Rago, Scott Fletcher, Lachlan Arentz, Lily Tuong, Linda Glowacki, Dimitri Gerostamoulos, Olaf H. Drummer 10:56 – 11:09 [DUI O-10] **Establishing per se limits in drug impaired driving cases: effects of raising the lower limit for different drugs** Gudrun Høiseth, Knut Hjelmeland, Jørg Mørland 11:09 – 11:22 [DUI O-11] **Comparison of the accuracy and precision of THC and cocaine/benzoylecgonine in whole blood when using single point, two point or multipoint calibration in relation to analysis of samples obtained under Section 5A of the Road Traffic Act 1988** Anita Tarnoska, Vincenzo Abbate, Susan C Grosse, Mark C Parkin 11:22 – 11:35 [DUI O-12] **The effects of sleepiness on the metabolome – road to biomarkers in oral fluid** Michael Scholz, Andrea E. Steuer, Akos Dobay, Stefan Lakaemper, Kristina Keller, Hans-Peter Landolt, Thomas Kraemer 11:35 – 11:48 [DUI O-13] **Effects of sampling & handling practices on preanalytical loss of nitrous oxide from whole blood samples** Angelica Ø Lindholm, Marie Katrine Klose Nielsen, Mette Kristensen, Brian Schou Rasmussen 11:48 – 12:01 [DUI O-14] **Driving under influence in Finland: Trends and remarks over the past ten years** Aino Kankaanpää, Petri Varjos, Nina Lauronen, Teemu Gunnar		

10:30 – 12:30	12:01 – 12:14 [DUI O-15] **Soft mobility and DUI-related traffic accidents: the results of a study conducted in Western Veneto in the years 2019 – 2023** Federica Bortolotti, Francesco Taus, Daniele Leonardi, Matilde Bissolo, Matilde Murari, Nadia M Porpiglia, Sara Pesavento, Anna Bertaso, Rossella Gottardo 12:14 – 12:27 [DUI O-16] **Changing gears in 2023 – putting the brakes on drugs in driving in New Zealand** Rosemary C A Moar, Helen A Poulsen, Matthew R Hosking	
12:30 – 13:15	Break and Exhibition	
13:15 – 13:45		**Flash Poster Presentation – Postmortem toxicology** Chair: Brigitte Desharnais 13:15 – 13:20 [PM F-P-01] **Redistribution of amlodipine in post-mortem samples** Jing Chang, Jian Huang, Xin Xin Ren, Yu jing Luan, Yun feng Zhang, Rui hua Wang 13:20 – 13:25 [PM F-P-02] **Gastric content analysis as a forensic tool in delayed pesticide toxicity deaths: A case series from South Africa** Bronwen Davies 13:25 – 13:30 [PM F-P-03] **Death by cannabis** Donata Favretto, Antonello Cirnelli, Cristina Basso, Maddalena Galeazzi, Fabio Mattiazzi 13:30 – 13:35 [PM F-P-04] **Anabolic steroids: "Deadly poisons"** Frédéric Aknouche, Christophe Marue-jouls, Claire Trebuchet, Kevin Fargeot, Fatima Korkmazyigit, Laureen Thion, Laurie Gheddar, Pascal Kintz 13:35 – 13:40 [PM F-P-05] **But did they drink it?** Sherri L. Kacinko, Tammy Y. Welch, Sharana D. Cook, Michael E. Lamb	**Flash Poster Presentation – Free topics** Chair: Islam Larabi 13:15 – 13:20 [DIV F-P-01] **Adverse analytical finding for ostarine in a doping context explained by contamination by body fluids** Jean-Claude Alvarez, Isabelle Etting, Islam Amine Larabi 13:20 – 13:25 [DIV F-P-02] **Evaluating occupational lead exposure in an Algerian lead battery recycling plant: Significance for worker health and safety** Faiza Bouchala, Sabah Benboudiaf, Anne Boos, Mohamed Hamadouche, Pascal Ronot, Islah El Masoudi, Mohamed Azzouz 13:25 – 13:30 [DIV F-P-03] **Be aware of (R)-methamphetamine: negative immunoassay vs. positive confirmation analysis** Jennifer Liut, Burkhard Madea, Michael Krämer, Alexandra Maas-Gramlich 13:30 – 13:35 [CT F-P-01] **Two-year report of pediatric unintentional cannabinoid intoxication in a Sicilian Pediatric Hospital** Francesca Di Gaudio, Davide Albano, Ilaria Calabrese, Sergio Indelicato, Vita Giaccone, Annamaria Cucina, Domenico Cipolla, Ettore Piro, Antonina Argo 13:35 – 13:40 [AM F-P-01] **Excretion of metabolites after oral ingestion of mitragynine** Josephine Krauskopf, Cecilia Henrich, Elisabeth Prevete, Johannes T. Reckweg, Eef L. Theunissen, Kim P. C. Kuypers, Johannes G. Ramaekers, Alexander Paulke, Stefan W. Toennes
13:45 – 14:00		
14:00 – 16:00	**Scientific Session 9 – Clinical toxicology** Chair: Naren Gunja, Hans Maurer 14:00 – 14:13 [CT O-1] **Characterization of the alcohol biomarker phosphatidylethanol in donor whole blood and apheresis red blood cells** Theresa Kinard, Loralie Langman, Paul Jannetto, Christine Snozek 14:13 – 14:26 [CT O-2] **Ayurvedic medicine: A 'natural' way of managing type 2 diabetes? A case study** Jenna Irion, Lubbe Wiesner 14:26 – 14:39 [CT O-3] **Accidental intoxication of three policemen with ADB-BINACA after inhaling substance containing dust** Wiebke Rudolph-Allritz, Catharina Baesler, Marc Villwock	

14:00 – 16:00	14:39 – 14:52 [CT O-4] **A case of acute psychosis post consumption of Delta-9-o THC and HHC laced edibles.** Rohan Rana, Shawn Bested, Brook Bested, Sumandeep Rana 14:52 – 15:05 [CT O-5] **Investigating γ-hydroxybutyrate (GHB) detection time and associated symptoms: Insights from a randomized clinical trial** Kathrine Bohn Faldborg, Lambert Kristiansen Sørensen, Jørgen Bo Hasselstrøm, Charlotte Uggerhøj Andersen 15:05 – 15:18 [CT O-6] **Investigating psilacetin metabolism: in vivo conversion to psilocin in male rats** Ana MF. Pego, Grant C. Glatfelter, David R. Manke, Andrew Chadeayne, Michael H. Baumann, Marta Concheiro-Guisan 15:18 – 15:31 [CT O-7] **Use of an untargeted screening workflow to detect new LSD metabolites following controlled LSD administration to humans** Sina Favrod, Friederike Holze, Matthias E. Liechti, Thomas Kraemer, Andrea E. Steuer 15:31 – 15:44 [CT O-8] **Two cases of intoxication with delta-9-tetrahydrocannabiphorol (THCP), a new semi-synthetic cannabinoid.** Marie Carles, Lucas Bertaina, Anne-Laure Pelissier, Jean-Claude Alvarez, Romain Pelletier, Thomas Gicquel, Caroline Solas-Chesneau, Nicolas Fabresse 15:44 – 15:57 [CT O-9] **Glucagon like peptide-1 analogues and misuse for weight-loss purposes: semaglutide and liraglutide proteins analysis in whole blood samples by the use of LC-ESI-HRMS method** Nadia Arbouche, Alice Ameline, Laurie Gheddar, Jean-Sébastien Raul, Pascal Kintz		
16:00 – 16:30	Break and Exhibition	Poster gallery – HR F-P-1 to P-11	Poster gallery – DIV F-P-1 to P-32
16:30 – 18:00	**Scientific Session 10 – Analytical methods** Chair: Simon Elliott, Dirk Wissenbach 16:30 – 16:42 [NAA O-3] **Evaluation of spectroscopic techniques for on-site drug testing on festival seizures** Natalie Meert, Joy Eliaerts, Filip Van Durme, Sarah M.R. Wille, Nele Samyn 16:42 – 16:54 [AM O-2] **Ketamine – a new (or old) kid on the block: A comprehensive three-year spatio-temporal study in Belgium through wastewater-based epidemiology** Natan Van Wichelen, Tim Boogaerts, Maarten Quireyns, Rania Dermitzaki, Peter Delputte, Sofie Schaerlaekens, Naomi De Roeck, Bavo Verhaegen, Koenraad Van Hoorde, Hadrien Maloux, Veronik Hutse, Celine Gys, Adrian Covaci, Alexander L.N. van Nuijs 16:54 – 17:06 [AM O-3] **Insights from four years of wastewater surveillance for novel psychoactive substances from up to 20 countries** Richard Bade, Dhayaalini Nadarajan, Nikolauos Rousis, Sangeet Adhikari, Christine Baduel, Lubertus Bijlsma, Erasmia Bizani, Tim Boogaerts, Daniel Burgard, Sara Castiglioni, Andrew Chappell, Adrian Covaci, Erin M Driver, Fernando Fabriz Sodre, Despo Fatta-Kasinos, Aikaterina Galani, Cobus Gerber, Emma Gracia-Lor, Elisa Gracia-Marin, Rolf U Halden, Ester Heath, Carolin Huber, Felix Hernandez, Julia Huchthausen, Emma Jaunay, Foon Yin Lai, Heon-Jun Lee, Maria Laimou-Geraniou, Jandyson Machado Santos, Vera Ocenaskova, Jeong-Eun Oh, Kristin Olafsdottir, Temilola Oluseyi, Kaitlyn Phung, Marco Pineda Castro, Magda Psichoudaki, Xueting Shao, Arndis Sue Ching Love, Rafael Silva Feitosa, Cezar Silvino Gomes, Bikram Subedi, Nikolaos Thomaidis, Diana Tran, Alexander L.N. van Nuijs, Taja Verovsek, Degao Wang, Jason M White, Viviane Yargeau, Ettore Zuccato, Jochen Mueller 17:06 – 17:18 [AM O-4] **Comparison of four experimental setups for bias and precision studies in method validation according to different guidelines exemplified for a LC-MS/MS assay for morphine, hydromorphone and their metabolites in human plasma** Lisa Oßowski, Daniela Wissenbach, Frank T. Peters 17:18 – 17:30 [AM O-5] **Mathematics-based best practices for the method of standard addition** Brigitte Desharnais, Étienne Lebrun, Jocelyn V Abonamah, Szabolcs Sofalvi 17:30 – 17:42 [AM O-6] **Development of a sensitive SPE-LC-MS/MS method for analysis of insulin variants, C-peptide and hemoglobin in postmortem vitreous humor and cerebrospinal fluid** Hans Brabec, Jörg Röhrich, Cora Wunder 17:42 – 17:54 [AM O-7] **Wastewater-based epidemiology for measuring illicit drug use: increased evidence for early-warning type of information and correlation between different indicators for drug use** Teemu Gunnar, Aino Kankaanpää		
18:00 – 22:00	Vendor Workshop – Biotage		

FRIDAY, 6TH SEPTEMBER 2024

08:30 – 10:00

Scientific Session 11 – Postmortem toxicology

Chair: Lana Brockbals, Dimitri Gerostamoulos

08:30 – 08:42 [PM O-1]
Ethanol production in the gut: An autopsy case
Maiko Kusano, Chikara Kohda, Masaya Fujishiro, Taka-aki Matsuyama

08:42 – 08:54 [PM O-2]
Drug-related deaths at Australian music festivals
Jennifer L. Schumann, Riyad Santamarina, John Fitzgerald, David Caldicott

08:54 – 09:06 [PM O-3]
Death of an infant due to the administration of diphenhydramine and zopiclone
Silvana Petzel-Witt, Stefan W. Toennes, Constanze Niess

09:06 – 09:18 [PM O-4]
Further evaluation of bladder wash as an alternative specimen for postmortem toxicology: comparison to screening results of urine and kidney tissue
Andrea E. Steuer, Sandra N. Poetzsch, Stephan A. Bolliger, Thomas Krämer

09:18 – 09:30 [PM O-5]
Forensic Entomotoxicology: Assessing the potential of necrophagous larvae for post-mortem toxicological analysis, using diazepam in a minipig model
Olwen Charlotte Groth, Anaëlle Pi, Andres Eskjær Jensen, Susan Rahaus, Gabriele Roider, Matthias Graw

09:30 – 09:42 [PM O-6]
Drug identification through analysis of maggots recovered from skeletonized dead body – A case study
Rida Akbar, Muhammad Imran, Muhammad Taimoor Chaudhary, Saima Afzal, Muhammad Irfan Ashiq, Muhammad Amjad

09:42 – 09:54 [PM O-7]
Post mortem distribution of isotonitazene and its three metabolites in the first lethal case observed in France
Jean-Joseph Bendjilali-Sabiani, Juliette Descoeur, Maisy LOSSOIS, Céline EIDEN, Hélène PEYRIERE, Olivier MATHIEU

10:00 – 10:30 Break and Exhibition | Poster gallery – PM F-P-1 to P-48 | Poster gallery – AB P-1 to P-12

10:30 – 12:30

Scientific Session 12 – Novel psychoactive substances II

Chair: Volker Auwärter, Svante Vikingsson

10:30 – 10:43 [NPS O-10]
In vitro structure-activity relationships and forensic case series of emerging 2-benzylbenzimidazole opioids
Liam M. De Vrieze, Sara E. Walton, Eline Pottie, Donna M. Papsun, Barry K. Logan, Alex J. Krotulski, Christophe P. Stove, Marthe M. Vandeputte

10:43 – 10:56 [NPS O-11]
New wine in old skins: MDMB-BINACA and its detection, metabolism and pharmacology
Martin N. Scheu, Annette Zschiesche, Benedikt Pulver, Volker Auwärter

10:56 – 11:09 [NPS O-12]
In vitro neurotransmitter inhibition of recently detected synthetic cathinones 2-methyl-alpha-PiHP, N-butyl-butylone, and N-cyclohexyl-butylone
Manuela C. Monti, Maria Wikström, Mattias Persson, Henrik Gréen

11:09 – 11:22 [NPS O-13]
In vitro γ-aminobutyric acid type A receptor activity of prescription and novel benzodiazepines detected on the European illicit drugs market
Caitlyn Norman, Nina Ottosson, Amaia Jauregi-Miguel, Sara I Liin, Henrik Gréen

11:22 – 11:35 [NPS O-14]
Synthesis and functional evaluation of synthetic cannabinoid receptor agonists related to ADB-HEXINACA
Eric Sparkes, Axelle Timmerman, Jack W. Markham, Rochelle Boyd, Rebecca Gordon, Katelyn A. Walker, Richard C. Kevin, David E. Hibbs, Samuel D. Banister, Elizabeth A. Cairns, Christophe P. Stove, Adam Ametovski

11:35 – 11:48 [NPS O-15]
Short- and long-term stability of synthetic cathinones and dihydro-metabolites in human whole blood and urine samples
Abdulaziz A. Aldubayyan, Erika Castrignanò, Simon Elliott, Vincenzo Abbate

11:48 – 12:01 [NPS O-16]
Metabolism study of 3-chloromethcathinone (3-CMC) by dried blood spot (DBS) sampling after controlled administration using a murine model
Serena Mestria, Sara Odoardi, Valeria Valentini, Giulia Biosa, Giorgia Corli, Marta Bassi, Matteo Marti, Sabina Strano Rossi

10:30 – 12:30	12:01 – 12:14 [NPS O-17] **3-CMC, 4-CMC, and 4-BMC metabolic profiling in humans: New major metabolic pathways to document consumption of methcathinone analogues** Jeremy Carlier, Diletta Berardinelli, Omayema Taoussi, Gloria Daziani, Francesco Tavoletta, Piotr Adamowicz, Francesco Paolo Busardò 12:14 – 12:27 [NPS O-18] **Assessment of α-PVP disposition in oral fluid, sweat and urine in humans by GC-MS/MS analysis** Nunzia La Maida, Annagiulia Di Trana, Georgina de la Rosa, Clara Pérez-Maña, Esther Papaseit, Lourdes Poyatos, Manuela Pellegrini, Alessandro Di Giorgi, Magí Farré, Simona Pichini		
12:30 – 13:15	Break and Exhibition		
13:15 – 13:45		**Flash Poster Presentation – NPS II** Chair: Caitlyn Norman 13:15 – 13:20 [NPS F-P-06] **Oral fluid insights: Unveiling trends in illicit substance consumption and new psychoactive substances at Brazilian festivals through oral fluid analysis** José Luiz Costa, Aline F. Martins, Náthaly C. B. F. dos Santos, Marcela de Oliveira Soares, Bruna S. D. de Oliveira, Paula Christiane Soubhia, Rafael Lanaro 13:20 – 13:25 [NPS F-P-07] **Protonitazepyne and metonitazepyne pharmacological profiling: Assessment of μ-, κ-, and δ-opioid receptors activation with a novel high-throughput non-radioactive GTP Gi binding assay** Diletta Berardinelli, Simona Zaami, Benedikt Pulver, Francesco Paolo Busardò, Volker Auwärter 13:25 – 13:30 [NPS F-P-08] **Deschloroketamine derivatives: Studies on their in vivo and in vitro metabolism and their microbial biotransformation in wastewater by means of hyphenated mass spectrometry** Fabian Frankenfeld, Lea Wagmann, Anush Abelian, Jason Wallach, Adeboye Adejare, Simon D. Brandt, Markus R. Meyer 13:30 – 13:35 [NPS F-P-09] **Identification of hexahydrocannabiphorol metabolites in human urine** Willi Schirmer, Stefan Schürch, Wolfgang Weinmann 13:35 – 13:40 [SOHT F-P-06] **First evidence of protonitazene, a novel synthetic opioid, in human hair** Pascal Kintz, Laurie Gheddar, Nadia Arbouche, Simona Pichini, Frédéric Aknouche, Christophe Maruejouls, Alice Ameline	**Flash Poster Presentation – Free topics II** Chair: Jennifer Schumann 13:20 – 13:25 [HR F-P-02] **Application of an untargeted metabolome approach to screen for biomarkers of nitrite poisoning** Marta Massano, María Cobo-Golpe, Thomas Kraemer, Andrea E. Steuer 13:25 – 13:30 [DUI F-P-01] **Occurrence of semi-synthetic cannabinoids in seized cannabis products from Eastern Denmark** Christian Falck Jørgensen, Brian Schou Rasmussen, Kristian Linnet, Ragnar Thomsen 13:30 – 13:35 [DUI F-P-02] **New psychoactive substances and psycoactive pharmaceuticals assessment in drivers' oral fluid samples** Miriam Blanco-Ces, Rosa Montes-Goyanes, Elena Lendoiro, María Cobo-Golpe, Ángela López-Rabuñal, Angelines Cruz-Landeira, Manuel López-Rivadulla, Ana de-Castro-Ríos 13:35 – 13:40 [DUI F-P-03] **Analyzing cannabinoids in blood and oral fluid: Implications for drug-impaired driving** Curt E. Harper
13:45 – 14:00			

14:00 – 15:00	**Scientific Session 13 – Free topics** Jean-Claude Alvares, Barry Logan 14:00 – 14:12 [DIV O-1] **Epidemiological profile of pregabalin misuse and abuse in the region of Ouargla, Algeria** Amine Mohamed Kerdoun, Anissa Zergui, Assia Yamoun 14:12 – 14:24 [DIV O-2] **Structure-activity relationship of P-gp ATPase modification by synthetic cannabinoids and drug-drug interaction prediction** Lea Wagmann, Anna Spanier, Markus R. Meyer 14:24 – 14:36 [DIV O-3] **Amino acids and their metabolites as biochemical markers of putrefaction** Laura Franke, Hannah Ihle, Kristina Rieger, Viviane Stammer, Senta Niederegger, Dirk K. Wissenbach, Frank T. Peters, Gita Mall 14:36 – 14:48 [DIV O-4] **Mitragynine and 7-hydroxymitragynine plasma pharmacokinetics after controlled oral mitragynine isolate administration to healthy human subjects** Marilyn A. Huestis, Martin A. Brett, John Bothmer, Thomas Hudzik, Jack E. Henningfield, Ramsey Atallah 14:48 – 15:00 [DIV O-5] **Characterization of 'prophetic' nitazenes: insights from in vitro μ-opioid receptor assays and in vivo behavioral studies in mice** Marthe M. Vandeputte, Grant C. Glatfelter, Donna Walther, Nathan K. Layle, Danielle M. St. Germaine, István Ujváry, Donna M. Iula, Michael H. Baumann, Christophe P. Stove
15:00 – 15:15	Short Break
15:15 – 18:00	TIAFT Annual General Meeting

19:30	Conference Dinner and Awards Ceremony

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Scientific Session 1 – Applications of high resolution mass spectrometry

8:30 – 10:00 Tuesday, 3rd September, 2024

Chair: Ilkka Ojanperä, Daniel Pasin

HR O–1

Feasibility of using routine forensic toxicology data for metabolomics studies exemplified for amphetamine

Annina Bovens, Claudio Leu, Thomas Kraemer, Andrea E. Steuer
Department of Forensic Pharmacology and Toxicology, Zurich Institute of Forensic Medicine, University of Zurich, Zurich, Switzerland

Background & Aims: Metabolome studies focus on the measurable change in (endogenous) metabolites triggered by a certain stimulus. In forensic toxicology, it is of interest to find analytical biomarkers for xenobiotics like drugs of abuse (DOA) that can improve case interpretation or elucidate underlying pharmacological mechanisms. (Placebo-) controlled clinical studies represent the gold standard for metabolome investigations but are hardly possible for DOA in humans for ethical reasons. This raised the question of whether routine cases could be used for this purpose. In forensic toxicology, untargeted workflows have become common, so there is the possibility of re-processing routine data files retrospectively with metabolomics-like workflows. However, several challenges have to be considered. For routine cases, drug doses and time of ingestion remain unknown and confounding factors cannot be controlled, leading to some variability. When re-evaluating data files, additional inter-batch differences are introduced during preparation and data acquisition. This is particularly important as untargeted metabolome comparisons are only made based on peak area differences (metabolites of interest are unknown before analysis omitting quantification). Therefore, it is necessary to evaluate whether using such (retrospective) routine data for metabolomics studies in a forensic toxicological context is possible. Here, we aimed to compare (endogenous) metabolomic findings of a placebo-controlled amphetamine administration study in humans (A) to routine cases positive and negative for amphetamine, prepared and analyzed within a single analytical batch ("one-batch", B) and after re-evaluation of data files prepared and analyzed over 6 months ("multiple-batch", C).

Methods: For the controlled study (A), plasma was collected from healthy volunteers (n_{pos}=18, n_{neg}=18) 3.5 h after crossover administration of amphetamine (single 40 mg dose) and placebo (Holze 2019). For the routine data set, whole blood from selected cases was used (n_{pos}=36, n_{neg}=35), matching in age and drug co-administration. This data set was once analyzed and evaluated as "one-batch" (B) and once as "multi-batch" (C), measured over 6 months. All samples were extracted via protein precipitation (acetonitrile, 1:3, v/v) and analyzed with an untargeted liquid chromatography-tandem mass spectrometric method (Bruker Elute UHPLC; Bruker Impact II TOF-HRMS; data-independent acquisition; ESI+). Peak picking and alignment were done simultaneously for A, B, and C with MS-DIAL (version 4.9). In MetaboAnalyst (version 5.0), PQN-normalization was conducted, while further statistical analyses were performed in R (version 4.3.2). Features defined by a specific combination of mass and retention time were evaluated for significance by comparing amphetamine-positive and negative cases for A, B, and C separately. Features fulfilling the following criteria were considered significant: foldchange (fc) 0.5 or fc 2, p-value 0.05, present in 80% of the cases per batch and peak area 1000.

Results & Discussion: When systematically comparing metabolome results between a controlled study (A) and routine cases analyzed under two different conditions ("one batch", B, and "multi-batch", C) we would expect significant features from A to be found in B and C as well. First, significant features in A were defined as the basis for comparison with B. 405 (92%) significant features from A were also present in B; however, only 15 (3.5%) of them were significant. These matching and significant features are all suspected to be related to amphetamine (fragments) based on library matches. As for endogenous metabolites, most features in B showed much smaller differences between amphetamine-positive and -negative cases, as most p-values were around 0.1 and/or fcs around 1.1. Therefore, feature-based uni-variate statistical testing seems unsuitable for endogenous biomarker searches with small differences between treatment groups. However, while significantly changed features from A did not match the results from B, 497 other features still met the defined significance criteria. It cannot be excluded that changes in these additional B features were caused by amphetamine, considering presumably higher and repeated dosing in routine cases compared to the controlled study, or they simply occurred by chance. Secondly, significant B features were compared to C. All significant B features were present in C, but only 46 (10%) were significant. Features insignificant in C showed p-values around 0.2 and/or fcs around 0.75, pointing out the relevance of inter-batch differences regarding data acquisition.

Conclusion: We aimed to evaluate the utility of (retrospective) routine data in metabolomics, particularly in forensic toxicology. The comparison between a controlled study (A) and routine cases (B and C) revealed several key findings. For the current dataset (n=71 routine cases), only few significantly changed features from A could be confirmed in routine cases (B). Larger sample sizes or more sophisticated multi-variate statistics might compensate for higher inter-individual variation in routine cases and presumably allow a better overlap. As expected, re-evaluating retrospective routine data introduces even greater variation (B vs. C). Nevertheless, drug metabolites could still be detected in routine cases (B and C). This confirms the general feasibility of (re)using routine cases or acquired HRMS datafiles for untargeted metabolome(-like) investigations if expected changes are high enough.

Characterizing consumption markers of the designer benzodiazepine bretazenil by high-resolution mass spectrometry using human hepatocytes and authentic postmortem samples

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Background & Aims: Benzodiazepines are traditionally used in clinical settings as anxiolytics, sedatives, or relaxants. However, designer benzodiazepines, in current times, have gained widespread notoriety as novel psychoactive substances (NPS), and are often abused alone or with other drugs, most notably opioids. This phenomenon known as "benzo-dope" can lead to debilitating health effects and serious morbidity. Designer benzodiazepines in some cases are disguised as therapeutic drugs and their administration may pose significant danger to unsuspecting persons. Currently, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) monitors 33 designer benzodiazepines since their appearance on the illicit market in 2007.

Bretazenil, a partial agonist of gamma-aminobutyric acid (GABA) receptors, albeit an old compound, re-surfaced in 2022 in Sweden and has subsequently been monitored by the EMCDDA. Although the abuse potential of bretazenil per literature is lower than for typical prescription benzodiazepines, its toxicity and likely concomitant abuse with other drugs necessitate the identification of consumption markers useful in clinical and forensic settings. The present study was carried out to detect and characterize markers of consumption of the largely unexplored metabolism landscape of bretazenil utilizing *in silico* prediction tools, incubation with hepatocytes, and authentic human biosamples from a postmortem intoxication case.

Methods: Open access *in silico* toolkits, GLORYx and Biotransformer (v. 3.0) were used to generate bretazenil phase I and II metabolites in humans. The threshold for GLORYx was set at 20% of the probability prediction score. Bretazenil was incubated with pooled cryopreserved hepatic cell lines for 3 h at 37 °C to assess its *in vitro* metabolism. Authentic human urine and blood from a bretazenil-positive postmortem case were analyzed for confirmation. Incubates and samples were mixed with acetonitrile and centrifuged, and supernatants were evaporated to dryness before reconstitution in 0.1% formic acid in water and 0.1% formic acid in acetonitrile (90:10, v/v); urine was prepared following enzymatic hydrolysis with β-glucuronidase (*P. vulgata*) and without hydrolysis. All samples were incubated and/or extracted along with negatives and/or blanks to monitor the experimental conditions.

Samples were analyzed with liquid chromatography-high resolution tandem mass spectrometry (LC-HRMS/MS) using reverse-phase chromatography with a biphenyl column and full scan and data-dependent acquisition in positive and negative ionization modes to comprehensively obtain the fragmentation pattern of bretazenil and its metabolites. Data mining was performed using Thermofisher Compound Discoverer (v. 3.1.1.12) to aid in annotating predicted and unpredicted metabolites in a targeted and untargeted workflow, respectively.

Results & Discussion: *In silico* prediction yielded eighteen and four metabolites from GLORYx and Biotransformer (v. 3.0), respectively, the main reactions being aromatic hydroxylation and further *O*-glucuronidation, sulfation, or methylation. After 3 h incubation with hepatocytes, eight metabolites were detected, mostly comprising hydroxylation at the pyrrolidine ring, but also with hydroxylation at the *tert*-butyl chain and the bromophenyl ring, reduction, carboxylation, glucuronidation, and cysteine conjugation. Similar metabolites with comparable intensity were identified in postmortem urine and blood, although the metabolite formed through hydroxylation at the pyrrolidine ring and reduction was preponderant (fifth most intense metabolite *in vitro*). Additional minor metabolites comprising glucuronidation or sulfation were detected in urine. The main metabolites in the authentic urine sample showed an intensity 5 to 10 times higher than that of bretazenil, but the parent compound was predominant in blood. Enzymatic hydrolysis did not significantly increase the signal of non-conjugated urinary metabolites.

Conclusion: The NPS market is constantly changing and presents new challenges for clinicians as well as forensic laboratories, thus identifying sensitive and specific markers of consumption is imperative. In this study, we comprehensively characterized the metabolism of the designer benzodiazepine bretazenil using *in silico* prediction toolkits, incubation with cryopreserved hepatic cell lines, and authentic urine and blood from a postmortem intoxication case. We propose the parent compound and the two most intense metabolites formed through pyrrolidine hydroxylation and a combination of pyrrolidine hydroxylation and reduction as markers of consumption of bretazenil. *In vitro* metabolism studies accurately simulated human biotransformation as observed in authentic urine and blood samples.

HR O-3

Development and optimization of compound identification scoring and data automation in high-resolution mass spectrometry analysis

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Background & Aims: In the context of extensive toxicological drug screening, it is crucial to establish stringent identification criteria to accurately detect all substances, including drugs of abuse, therapeutic agents, poisons, and novel psychoactive substances. This becomes pivotal for laboratories regularly handling a growing volume of medico-legal deaths, cases involving drug-facilitated crimes (DFC), and instances of drug-impaired driving (DUID). The efficient processing of results is essential to substantially reduce the resources needed for generating high-quality reports in forensic settings.

This study aimed to optimize data automation within instrument software to assist in data processing and reporting of acquisition results from the High-Resolution Mass Spectrometry Analysis (HRMS) technique, Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry (LC-QTOF-MS) with Sequential Window Acquisition of All Theoretical Fragment Ion Spectra (SWATH). The final objective was to determine the most efficient, sensitive, and specific identification criterion and automation techniques at both the limits of detection and higher concentration ranges of the targeted analytes.

Methods: To optimize the combined weight score (CWS) and qualitative data identification criteria, twelve master mixes were prepared using reference materials in methanol. Each mix were spiked into blood and urine blanks at four concentrations resulting in a total of 24 sample mixes, injected in duplicate. HRMS data were collected using a QTOF in full-scan positive ion mode with SWATH acquisition. Full-scan data were collected at a resolution of ~30,000 (at m/z 300) and product ion data were collected at ~15,000. Data were analyzed using SCIEX OS software and analyzed using laboratory generated flagging rules and custom calculations within the software. A CWS was generated with weighted summation of these 4 parameters: mass score, retention time score, isotope pattern score, and library score. Data matrix analysis was mirrored and modified by a previously published experimental design of optimizing HRMS data analysis parameters. Based on the identification results, CWS were assessed for rates of true positives (TP), false positives (FP), false negatives (FN) and true negatives (TN). The total number of TP, FP, FN, TN were obtained and used to calculate efficiency, sensitivity and specificity for each set and threshold. For applicability, the CWS was tested against 65 previously extracted authentic case samples in blood and urine.To optimize custom columns and conditional look up tables, rigorous testing of previously acquired casework samples was conducted. The processing method includes a "calculated columns" capability, employing Excel-style formulas to create custom columns used to filter, parse, calculate and format data. Parsing of the data begins with various lookups using the 'Sample ID' to determine the appropriate processing pathway and bracketing of quality controls. The sections built and optimized included acceptability and flagging for the limit of detection, retention time, relative retention time, quality controls, and internal standards. Additional flags were built for dilution considerations, detector saturation, monitored components, and components requiring special interpretation. Previously analyzed case samples were reprocessed to assess applicability.

Results & Discussion: Custom calculated columns including a total of 280 individual calculations were created by our laboratory using the coding programming available within the new features of the software, and subsequently divided into 18 sections with 51 subsections for organizational purposes. A total of 21 conditional lookup tables were also built, along with custom flagging rules specific to qualitative flagging of QTOF data: mass error, retention time error, isotope pattern and library score. These criteria were weighed and combined to provide an overall CWS. The CWS chosen resulted in a sensitivity of 87%, specificity of 97% and efficiency of 97%.

Although HRMS based technologies have made their way into forensic toxicology testing regimes, use of the technique is relatively novel and largely limited to screening, with other tandem-based technologies used as confirmation. The extensive data generated by HRMS techniques constrains their routine adoption in forensic testing.

Therefore, the development and optimization of data analysis parameters were essential during a multi-phase HRMS validation project aimed at developing a comprehensive and efficient method that can be used for both screening and confirmation. The use of custom calculations, flagging rules and conditional lookup functions has automated data processing systematically, and minimized transcription error, human error, and improves efficiency.

Conclusion: This study showed that although efficient data interpretation in the context of SWATH acquisition poses a considerable challenge due to its untargeted approach that subsequently results in the generation of exceptionally large data files, efficient data processing and automation for routine testing using SWATH is feasible. Such data processing workflows can be adopted by other laboratories facing similar challenges.

Postmortem metabolomics and the application of machine-learning to predict postmortem interval

HR O-4

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Background & Aims: Determining the postmortem interval (PMI) is critical in forensic investigations yet remains challenging due to diverse factors influencing postmortem changes. This study aims to leverage advanced analytical techniques, utilising high-resolution mass spectrometry (HRMS), and employ machine-learning algorithms to evaluate the potential of using postmortem metabolomics to predict PMI.

Methods: A pilot study analysed 4282 postmortem cases from July 2017 and November 2020, originating from five major cause-of-death groups. The inclusion criteria on PMI was either a confirmed death date or an estimated death date with < 48-hour deviation between "body found" and "last seen alive". HRMS data was retrospectively collected from our database of femoral blood toxicological screening were retrospectively collected. Principal component analyses (PCA) identified trends and outliers. Machine-learning models were used, with both random forest (RF) and artificial neural network (ANN), with data were divided into a training set (80%), test set (10%), and validation set (10%). Statistical modelling was performed in R and Python. An expansion of this study is planned to include 7429 cases from a more heterogenous cohort, with no specified cause-of-death criteria.

Results & Discussion: Here we present the preliminary results of the pilot study. PCA modelling revealed trends in feature differences with increasing PMI. Current PMI predictions for RF resulted in a mean absolute error (MAE) = 1.9 days with an R^2 = 0.39, and ANN resulted in a MAE = 1.4 days with an R^2 = 0.67. The ANN model was optimised by selecting the top 200 chromatographic features using feature selection via correlation and mutual information for linear and non-linear relationships, respectively. The optimised ANN model resulted in a MAE = 1.4 days with an increase in R^2 = 0.70. Further optimisation will be conducted prior final modelling with the validation set. At present, ANN shows the most promise with greater R^2 values and lower error margins, when compared to RF. These will be further evaluated with testing in the validation set. In addition, the models with be applied to the larger cohort of 7429 cases.

In Sweden, routine toxicological screening is conducted on all forensic autopsies at the central laboratory located at the Department of Forensic Genetics and Forensic Toxicology. This provides a substantial database of HRMS data, readily accessible for reuse in postmortem metabolomics investigations. One limitation to note is that the metabolome coverage is limited to analytical window of the routine toxicological method, rather than a method optimised specifically for metabolomics. Nevertheless, the key strength of using this database is that it eliminates the need for reanalysis and presents future opportunities for easier implementation into routine procedures.

Conclusion: Accurately predicting PMI is a highly sought-after question within forensic medicine. This study highlights the potential of postmortem metabolomics and machine-learning techniques in predicting PMI. Preliminary results, particularly with ANN, show promise, and with further optimisation and evaluation in a larger cohort we hope to improve predictive accuracy.

Data analysis workflows utilizing SQL for UHPLC-orbitrap and ion trap MS data in untargeted metabolomics

HR O-5

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Background & Aims: Liquid-chromatography-high-resolution mass spectrometry (LC-HRMS) is a powerful tool used for comprehensive screening of complex samples, encompassing compounds with diverse physiochemical characteristics. It is therefore an ideal choice for untargeted screening across various domains, including metabolomics for the discovery of new biomarkers. Traditional untargeted metabolomic studies prioritize highly abundant and frequently detected compounds. Full MS scans are acquired on individual samples since untargeted metabolomics requires a high number of scans per peak for feature definition, with fragmentation data usually extracted from pooled samples. However, this poses challenges in identifying exogenous features not consistently present in all or most samples.

With recent advancements in HRMS techniques, it is now possible to acquire fragmentation data for each sample without compromising the mandatory full MS scan frequency, thereby enhancing the likelihood of identifying significant features. However, current data analysis workflows in metabolomics do not incorporate within-sample fragmentation data for compound identification. Furthermore, the data analysis process in metabolomics typically employs either vendor-specific or open-source software, that does not scale beyond a certain threshold of samples without implementing data reduction steps.

This study therefore aims to develop an SQL-based data analysis workflow for LC-HRMS data with multiple detectors. This includes the development of *i*) open-source programming scripts to parse all relevant LC-(HR)MS data layers directly from vendor files to an SQL database, *ii*) basic LC-(HR)MS queries from the SQL database, including extracted ion chromatograms, MS/MS spectral comparisons, and grouping of MS/MS spectra. In addition, *iii*) MS/MS spectra from an orbitrap and ion trap detector, with identical collision settings are compared.

Methods: The data used in this study originated from a UHPLC-HRMS setup employing two chromatographies specifically developed for analyzing serum samples in untargeted metabolomics studies. The ID-X instrument (Thermo Fisher Scientific) utilized in this setup includes both an orbitrap and a dual-pressure ion trap. This configuration makes it possible in the metabolomics data acquisition workflow for each sample to be analyzed by full MS in the orbitrap, and MS/MS spectra are acquired at batch-level in the orbitrap. In addition, the ion trap is used for the acquisition of quick data-dependent MS/MS spectra in each sample while the orbitrap is performing full MS.

The open-source programming language Python in combination with the vendor direct-link library MSFileReader were used to access the data files. Selected information from the data files was parsed to an SQL database, and data was subsequently queried from this database. Vendor software (Freestyle, Thermo Fisher Scientific) was used to assess the completeness of parsed information.

Results & Discussion: A Python script was developed to extract selected information from RAW files acquired on serum samples using the ID-X instrument, utilizing commands specified in MSFileReader. This script directly parsed mass spectral data from the RAW files to a SQL database. The included information is mass, intensity, scan number, MS level, detector, retention time, precursor ion, file name, batch-related information, and selected mass spectrometer settings. The first three items listed were stored in one table, while the others were stored in another, linked by scan number and data file-related information for each sample. These tables were used to create an SQL database. Storing data in an SQL database allows for scalable retrospective data analysis, and fast access to large amounts of historic data, without a reduction in the different data layers.

With the data stored in the SQL database, mean-shift clustering was applied to group MS/MS spectra from the two mass detectors originating from the same precursor ion. Different approaches were tested to compare the orbitrap and ion trap spectra. Generally, the orbitrap and ion trap data corresponded well, disregarding the mass accuracy of the ions. The stored data allows tailored, multi-layered queries for ions in MS and MS/MS spectra, which is particularly useful for retrospective data analysis of larger LC-HRMS data sets.

The final script is available on GitHub (https://github.com/Pjheinsvig/orbitrapDB) and can be utilized by others with the same instrumental setup in their studies.

Conclusion: The study presents SQL-based data analysis workflows for LC-(HR)MS data from an untargeted metabolomics setup comprising orbitrap and ion trap data.

Our setup is well-suited for studies involving numerous data files, without the need for either data reduction or data inflation. The presented data analysis workflows are designed for untargeted metabolomics, but can easily be transferred for large-scale retrospective data analysis of forensic drug screening data from orbitrap-based screening methods.

Drug concentration prediction in forensic toxicology utilizing high-resolution QTOF tox screening

HR O-6

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Background & Aims: Forensic toxicology laboratories often use high-resolution mass spectrometry to screen for drugs in postmortem samples. We investigate the potential of using spectral data to predict drug concentrations, utilizing drug related features such as adducts, isotopes and metabolites. Our study aims to correlate spectral information obtained from ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC-QTOF) with quantitative results from UHPLC-QQQ, analyzing around 20,000 samples in order to investigate trends and outliers.

Methods: UHPLC-QTOF data together with quantitative results from UHPLC-QQQ from ~ 20,000 postmortem samples, are included in this study. QTOF data were processed using XCMS and correlated with drug concentrations using partial least square (PLS) analysis. Approximately 4/5 of the sample population were used to develop and optimize the multivariate model while the remaining 1/5 were used as a validation set enabling a full validation of the multivariate model. An extra 4000 prospective samples were used to test the model in a real setting.

Results & Discussion: PLS analysis was conducted on a subset of the sample population, encompassing 4,000 autopsy cases involving 7 analytes (acetaminophen, alimemazine, alprazolam, amphetamine, cocaine, oxycodone, and pregabalin), revealing high predictive power and robustness. Strong correlations between known and predicted concentrations (R2=0.70-0.99) were observed, and the correlation between predicted and observed concentrations closely aligns with inclinations close to 1 (0.9-1.2). Validation samples exhibited predicted concentrations within ± 50% of observed concentrations for 84-96% of cases, varying by drug. Notably, incorporating additional features such as drug isotopes, adducts, and metabolites enhanced model performance. Samples with poor quality such as coagulation or lipemia showed greater differentiation between predicted and observed values. Further evaluation will encompass a total of 21 analytes and 20,000 samples, with application to a larger prospective test set comprising approximately 4,000 cases.

Conclusion: In conclusion, our study highlights the potential of using high resolution screening data for predicting drug concentrations in forensic toxicology, which offers a rapid method for preliminary results and a possibility for quality assurance. Further investigation with additional analytes and samples is ongoing, including testing on a larger prospective dataset (~4,000 cases).

A metabolomics approach for CYP phenotyping: A proof-of-concept to use enzyme-based study designs

HR O-7

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Background & Aims: Cytochrome P450 (CYP) enzymes are vital in phase 1 drug metabolism. While the importance of these enzymes has been established, their inter-individual variation in activity remains a major challenge for clinical and forensic interpretation. Currently, an individual's enzyme phenotype is determined by administering established CYP probe substrates followed by determination of the pharmacokinetics of the metabolites – a standard that is unfeasible in forensic applications, especially postmortem. Finding endogenous biomarkers indicative of a CYP phenotype, e.g., by metabolomics techniques, could solve this persisting issue. Tightly controlled human in-vivo studies, e.g., administration of CYP-inhibiting or -inducing drugs, deliver the most promising samples to screen for endogenous CYP biomarkers. However, ethical, legal, cost, and/or complexity issues limit the possibility of human drug administration studies. Hence, it is necessary to find alternative experimental methods. One approach could be the use of human liver microsomes (HLM) or relevant isoenzymes that readily metabolize xenobiotics. However, endogenous substrates (mixtures) need to be added to screen for unknown, endogenous CYP biomarkers. Our approach aims to investigate whether complex matrices, such as blood, can be used as a source of endogenous metabolites in enzymatic incubations. The primary aim was to establish that HLMs and isoenzymes work as expected even with human matrix present in the assay. Analysis of exogenous and previously postulated endogenous biomarkers for a particular CYP phenotype should be used to prove the approach's general potential to identify CYP-related changes in endogenous compounds, prior to an untargeted search for potentially new CYP activity biomarkers.

Methods: Established HLM assay protocols were followed for all experiments. In brief, assays consisted of HLMs or isoenzymes (2C19, 2D6, or 3A4), the CYP co-substrate NADPH (regeneration system: NADP+, G6P, G6PD), superoxide dismutase, and MgCl in phosphate buffer. Blood from EDTA tubes or a 50% mixture of blood and phosphate buffer was added as an endogenous substrate mixture in addition to exogenous substrates (omeprazole, dextromethorphan, and midazolam for CYP2C19, CYP2D6, and CYP3A4, respectively) if needed. Control samples without endogenous substrates were prepared using phosphate buffer. Negative controls were prepared either by substituting HLM/isoenzymes or the regeneration mix with buffer. Incubations (n=5 per condition) were performed at 37 °C for one hour. Afterward, 50 µL of ice-cold ACN was added, and 50 µL of supernatant was transferred to a conical vial after vortexing and centrifugation. All samples were analyzed using a scheduled MRM method (Sciex QTrap 5500) targeting exogenous and endogenous CYP substrates and metabolites, and an untargeted metabolomics method (Sciex TripleTOF 6600). Targeted data was processed using Multiquant, while untargeted data was processed with MS-DIAL (4.9.2 and 5.2.2). Resulting peak areas were further evaluated using R.

Results & Discussion: Comparing targeted data of HLM assays containing buffer and dextromethorphan, midazolam or omeprazole with those containing either 50% blood or blood only, showed slightly higher dextrorphan formation when blood was present while OH-midazolam and 5-OH-omeprazole formation were similar (t-test, p=0.001, p=0.286, p=0.942). A non-significant decrease in dextromethorphan could be observed while midazolam and omeprazole decreased significantly after incubation (t-test, p=0.058, p=0.0001, p=0.002). These results indicate that adding blood does not hinder enzyme performance. Considering previously postulated endogenous CYP biomarkers, an increase of (11,12)-EET (postulated to be formed by CYP2C19 from arachidonic acid) in HLM assays could be observed, while it was not present in negative controls. Additionally, a non-significant decrease of deoxycholic acid and cortisol (postulated endogenous substrates of CYP3A4) was found in the respective isoenzyme assays. This is in line with the literature and indicates that our assay does mimic in-vivo conditions. While serotonin (postulated biomarker formed by CYP2D6 from 5-methoxytryptamine) has been readily detected in all our assays, 5-methoxytryptamine was not observed. By evaluation of the untargeted data, we were able to establish a list of 313 (2C19), 35 (2D6), and 22 (3A4) mostly unknown features, which exhibit a significant difference between the CYP incubations and both negative conditions but no significant difference between both negative conditions, setting them up to be potential biomarkers for their corresponding CYP enzymes. Omeprazole/OH-omeprazole, dextromethorphan/dextrorphan, and OH-midazolam were tentatively identified among those features. These findings further indicate the suitability of our approach to screen for endogenous CYP phenotype biomarkers and provide the basis for follow-up studies to identify such biomarkers.

Conclusion: We were able to show that standard HLM and isoenzyme assays still perform as expected with the addition of human blood as a source of numerous endogenous metabolites. With our experiments we were able to show increases/decreases of classical substrates/metabolites as well as the emergence of newly formed endogenous biomarkers previously postulated. It is possible to find significantly altered features in our experiment data using an untargeted metabolomics approach, hinting towards potentially new biomarkers for CYP phenotyping. There is a need for further research into these approaches as well as suitable strategies for comparison with and confirmation of in-vivo results.

Scientific Session 2 – Novel psychoactive substances I

10:30 – 12:30 Tuesday, 3rd September, 2024

Chair: Matthew Hosking, Carmen Jurado

NPS O–1

Update from the EU Early Warning System on new psychoactive substances

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Background & Aims: A large number of new substances have appeared in Europe over the last 25 years, with new compounds continuing to be detected each year. Operated by the EU Drug Agency (EMCDDA), the European Union Early Warning System (EU EWS) on New Psychoactive Substances (NPS) has provided early warning and strengthened preparedness and response to public health threats caused by new drugs since 1997.

Methods: The EU EWS allows Europe to rapidly detect, assess, and respond to public health threats linked to NPS. Data collected and analysed include event-based data on seizures by law enforcement, collected samples submitted to drug-checking services and serious adverse events linked to NPS. These data are complemented by annual reports, which include aggregated data on seizures and biological samples, including those associated with poisonings.

Results & Discussion: At the end of 2023, the EMCDDA was monitoring around 960 NPS. The number of substances in circulation remains high, with approximately 400 previously reported NPS detected in 2022. While some are seized in small amounts and infrequently, some appeared to have gained a foothold on the market. In 2022, a record 30.7 tonnes of NPS were seized by EU Member States. This increase has been driven by a large increase in seizures of synthetic cathinones, in particular 3-CMC. Despite this, there are possible signs that the number of new NPS entering the market may be slowing. While an average of 50 new NPS were reported each year between 2016 and 2022, this fell by 50 % in 2023, to 26. Legislative controls in Europe and non-EU source countries appear to have contributed to a reduction in the number of new derivatives of some drugs, such as fentanyl. Nevertheless, drug producers continue to create new substances to avoid legal controls. Following the steep decline in fentanyl derivatives appearing in Europe since 2018, the equally potent nitazene opioids have replaced them. Since 2022, there has been an increase in reports of nitazene opioid detections and poisonings in parts of Europe, and particularly in Estonia and Latvia. In addition, in 2023, three localised outbreaks of poisonings caused by nitazenes mis-sold as heroin were reported by France and Ireland. The data also suggests a possible increase in the detection of fake medicines containing nitazene opioids. The developments in the nitazene market are occurring in the context of recent signals of other possible changes to the opioid market in Europe such as the emergence of 'tranq-dope' and 'benzo-dope'. New threats continue to emerge: alongside hemp adulterated with synthetic cannabinoids over the past few years, semi-synthetic cannabinoids (e.g. hexahydrocannabinol, HHC) have emerged on the EU market in the last few years. They appear to be made from naturally occurring cannabinoids, such as CBD, and are sold openly in a broad range of consumer product forms. These include vapes and edibles that may be particularly attractive to young people.

Conclusion: Currently, the NPS market is characterised by complexity and increased integration with the market for established controlled drugs The market is globalized, resilient, and highly dynamic making it difficult to disrupt. Nonetheless, the situation across Europe often differs greatly, with the threats in any one area shaped by global and local factors. Forensic and toxicological information sources are critically important for but there remains a need for investment in forensic, analytical and toxicological capacity. There is a need to continue to build, maintain, and strengthen early warning systems to support preparedness and timely response to emerging public health and social threats. The EU EWS plays a central role in supporting national- and EU-level preparedness and responses to NPS.

Current state and recent advances of HighResNPS for suspect screening of new psychoactive substances

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Background & Aims: HighResNPS (highresnps.com) is an online crowd-sourced mass spectral (MS) database for new psychoactive substances (NPS). It was developed by the Section of Forensic Chemistry at the University of Copenhagen who maintained this resource until its transfer to TIAFT in October 2023. It is now overseen by the NPS Committee. The aim of this presentation is to provide the TIAFT membership an update on the current state and future prospects of HighResNPS.

Methods: The HighResNPS database was developed using the ASP.NET web framework and operates off a central table which contains all entries in the database. Users can contribute to this database either manually through the web interface, or by importing their compounds using the Microsoft Excel template provided. The entire database is also provided as a Consensus library which reduces the database to only one entry per unique compound. This entry contains the product ions that were observed for that compound across all entries and are scored based on their frequency. The Consensus library is then made available for download in 7 formats compatible with software from different vendors. Deep learning, through the use of different algorithms, were developed to improve the suspect screening capabilities of the database including retention time prediction, MS/MS spectral prediction and prophetic NPS structure determination.

Results & Discussion: As of April 2024, HighResNPS has a total of 6,702 entries corresponding to 2,330 unique compounds, mostly comprised of NPS and their metabolites. Product ion data is available for 1,666 compounds. HighResNPS has continued to show steady growth in users, with a total of 420 users from 47 countries (compared to 129 users at the start of 2020). Membership comprises mainly forensic toxicology laboratories, but also wastewater-based epidemiology, customs and illicit drug analysis laboratories. The retention time prediction model and associated personalised databases are active for 24 user methods. This feature allows these laboratories to apply HighResNPS in suspect screening contexts more efficiently and has facilitated the detection of NPS outside

routine targeted testing. The utility of HighResNPS to the scientific community is evidenced by the metrics of the originating article by Mardal et al, which has over 65 citations since 2019. Forty of these have been since 2022, with a Field Citation Ratio of 7.5 (i.e. cited 7.5 times more than the average article in its field). HighResNPS has collaborated with leading researchers in the field of artificial intelligence. Firstly, the generative model, DarkNPS, was able to generate a library of over 8 million structures, with many compounds of the top predicted compounds appearing on the market. Secondly, a web server was developed to give users a graphical user interface for the MS/MS prediction model, NPS-MS (nps-ms.ca), providing a means for users to predict MS/MS spectra at 10, 20 and 40 eV. Strategies to incorporate these initiatives into the HighResNPS database are being investigated. Recent measures to enhance user experience and to facilitate membership administration include use of MailChimp, a Slack workspace for member communication and a shared Google Drive for files that cannot be stored within the database website itself.

Conclusion: HighResNPS continues to be a premier resource for the detection of NPS in suspect screening contexts. The development of artificial intelligence-driven projects has considerably reduced the financial and temporal costs at maintaining an ever-increasing scope for NPS. Strategies to facilitate interaction with the TIAFT NPS committee and TIAFT members are in development. The successful transfer of HighResNPS ownership and running costs from the University of Copenhagen to TIAFT secures the future of this valuable resource for the TIAFT community and other NPS-related fields into the future.

NPS O–3

Changing trends in synthetic cannabinoid receptor agonist (SCRA) use in Scottish prisons: detection, prevalence and modes of use

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Background & Aims: Synthetic Cannabinoid Receptor Agonists (SCRAs) are one of the largest groups of New Psychoactive Substances (NPS) and they have been widely detected in toxicology casework and drug seizures. SCRAs have been routinely encountered in samples seized as part of the Scottish Prisons Non-Judicial Drug Seizure Monitoring Project, operating since 2019. Recent studies have highlighted increased levels of complex polydrug use within Scottish prisons particularly in relation to benzodiazepines, which have been detected alongside SCRAs infused into paper and card.

In December 2021, the Scottish prisons introduced a policy for photocopying all incoming items of mail to counter the smuggling of drugs via this route. Within this study, the effects of these procedural changes on the prevalence of different SCRA sample formats are considered. Additionally, this study assessed the impact of recent international legislative changes on the SCRAs being detected and the impact of the availability of precursors and laboratory equipment, synthesis instructions or 'semi-finished SCRA kits' from online SCRA vendors on the prevalence of individual SCRA-type compounds.

Methods: Non-attributable samples seized by the Scottish Prison Service (SPS) were analysed as part of an ongoing drug seizure monitoring project. The items were recovered during person or cell searches or following detection of the possible presence of a controlled substance during the screening of incoming items (mostly mail) by prison staff using Ion Mobility Spectrometry (IMS). All samples considered suitable for analysis were anonymized by SPS staff and transferred to the Home Office licensed drug testing laboratory at the Leverhulme Research Centre for Forensic Science (LRCFS) at the University of Dundee, Scotland for analysis.

Qualitative analysis was performed using a 7820A gas chromatograph coupled to a 5977E mass spectrometer (Agilent technologies, Santa Clara, CA, USA). Injection mode: 1mL sample injection and 20:1 split, injection port temperature: 200°C, carrier gas: He, flow: 1 mL/min. Column: HP-5MS, 0.33 mm, 0.2 mm x 25 m (Agilent Technologies). GC oven: 80°C held for 3 minutes; 40°C/min to 300°C held for 11 minutes; transfer line of 295°C. The mass spectrometer is operated in electron ionization (EI) mode. Ionization conditions: 70eV in full scan mode (50-550 amu), ion source: 230°C, quadrupole: 150°C. Compound identification by gas chromatography mass spectrometry (GC-MS) was carried out by comparison of the compound retention times and mass spectra in seized samples to a reference standard of known origin.

Results & Discussion: Since 2019 the Scottish Prisons Non-Judicial Drug Seizure Monitoring Project has analysed 4304 samples from 3220 seizures, of which 1143 (26.6%) tested positive for one or more SCRAs. From 2019 to 2021, infused paper or card accounted for over 95% of all samples containing SCRAs; however, the implementation of mail photocopying procedures in the Scottish prisons in December 2021 led to a rapid decrease in detections of SCRAs in this format and a subsequent detection of SCRAs in powders, tablets, and vape pods. In 2023, vape

liquids/pods accounted for the majority of SCRA detections (45%) followed by tablets and powders (26%). Since January 2023, there has been an increase in detections of waxy- or putty-like samples often visually consistent with cannabis concentrates colloquially referred to as 'budder' or 'crumble'. However, only 17% of these samples tested positive for THC, with 50% testing positive for SCRA compounds such as MDMB-4en-PINACA.

In addition to evolving SCRA formats, increased polydrug detections including SCRAs were observed. In 2023, SCRAs were detected alongside anabolic-androgenic steroids (e.g. oxymetholone), cathinones (e.g. dimethylpentylone) and novel synthetic opioids (e.g. metonitazene) in 21% of samples compared to 1% of samples in 2019. Some of these drugs are not usually associated with smoking/vaping, so their harms are not fully understood, resulting in potential increased harm to individuals.

Furthermore, following the emergence of ADB-BUTINACA and the introduction of legislation by China in 2021 banning seven SCRA core structures, a decrease in the detection of MDMB-4en-PINACA in Scottish prison samples was observed throughout 2022. Since early 2023, co-detections of MDMB-4en-PINACA and its precursor MDMB-INACA have increased. Research suggests that the online availability of 'semi-finished SCRA kits' and instructions for the conversion of the 'semi-finished SCRA' compounds not covered by the Chinese ban (e.g., MDMB-INACA) into the desired controlled compound (e.g., MDMB-4en-PINACA), are likely to be responsible for this re-emergence

Conclusion: Following the implementation of mail photocopying in Scottish prisons, sample types in which SCRAs have been detected have diversified. In addition, this study has identified increased hazards associated with polydrug use, in particular from the vaping of substances not usually inhaled alone or with SCRAs (e.g., anabolic-androgenic steroids/cathinones/benzodiazepines). Furthermore, the online availability of 'semi-finished SCRA kits' has been identified as a potential source of the re-emergence of compounds such as MDMB-4en-PINACA to the Scottish prison market. Increased understanding of the changing drug market and the challenges of SCRA use helps inform strategies to reduce supply and mitigate harm.

Epidemiology of new psychoactive substances in relation to traditional drugs of abuse in clinical oral fluid samples collected in Sweden

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Background & Aims: New psychoactive substances (NPS) are life-threatening due to their unpredictable potency and toxicity. Knowledge about NPS epidemiology, especially in relation to traditional drugs of abuse, could therefore be lifesaving. The aim of this study was to generate new epidemiological insights into the consumer profiles of NPS-users in order to provide guidance in clinical situations.

Methods: Using retrospective investigation of liquid chromatography-high resolution mass spectrometry data, the presence of 138 NPS were retrospectively determined in 34,183 clinical oral fluid samples from 9468 patients from psychiatric and addiction care clinics in Western Sweden collected between 2019-2020. The NPS investigated included the majority of those reported by the European early warning system during 2019-2020 as well as older NPS that have remained in popularity. The correlation between NPS use and patient characteristics (e.g. age and gender), as well as NPS use (including number of different NPS used) and traditional drugs was investigated. Statistical analyses were performed using Fisher's exact test for contingency tables and Pearson correlation test for comparison of scale data between groups. Significance levels were adjusted for mass testing using the Bonferroni correction.

Results & Discussion: Fifty-eight different NPS were identified which amounted to a total of 618 findings. 2.1 % of the patients were positive for an NPS at at least one occasion. Male gender and age ≥25 years correlated positively with NPS use with p-values of 1.6×10^{-12} and 6.9×10^{-7}, respectively (Fisher's Exact Test with a significance level of 1.8×10^{-3} corresponding to $p<0.05$). There was a general association between co-use of traditional DoA and NPS (an average of 3.0 traditional DoA in samples positive for NPS compared to 1.5 traditional DoA in samples negative for NPS; $p = 1.8 \times 10^{-52}$, Pearson correlation test). Hence, only samples positive for traditional DoA were included in statistical analyses comparing correlations between NPS and individual traditional DoA. Several traditional drugs correlated positively with NPS use, including ketamine, cannabis, heroin, alprazolam, cocaine, MDMA, ketamine, methadone and clonazepam. Ketamine correlated positively with all NPS classes except for cannabinoid NPS and of all the ketamine positive samples, 14% contained NPS. Mitragynine, unlike other NPS, had a negative correlation with several traditional drugs. Use of cannabinoid NPS correlated with the lowest number of classes of traditional DoA.

Conclusion: NPS use should be expected also at higher ages but generally occur at a low frequency. NPS are commonly combined with traditional drugs which can increase the risk for severe adverse effects. Particularly use of ketamine stands out as an indicator for NPS use. Correlating the use of NPS with traditional drugs show specific patterns of use that reflect the drug specific behaviour of the patients.

NPS O–5

Harm caused by metonitazene in New Zealand during 2022 and 2023

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Background & Aims: ESR undertakes "Border to Grave" drugs analysis for New Zealand. Analyses are undertaken on seizures from airports, mail centres, drug checking organisations (these are publicly funded legal organisations that allow people who use drugs to have their substances checked), as well as domestic drug seizures (New Zealand Police and Customs). ESR also undertakes analyses of biological samples associated with drug related deaths (coronial cases) and crimes as well as impaired drivers. The data generated from all these sources are combined to provide information of what drugs are entering New Zealand and what drugs are causing harm, where and when. This information assists High Alert, New Zealand's Drug Early Warning system, to provide information to the public to help reduce harm in communities, as well as issuing alerts and notifications for particularly dangerous or contaminated drugs.

In October 2022 metonitazene was first detected in New Zealand in a yellow powder associated with a death. Within a week of this death, High Alert issued a notification to interested organisations as well as public health authorities and people who use drugs that metonitazene was found in NZ in a yellow powder. Metonitazene is a derivative of benzimidazole opioids and was first reported internationally in a seized drug powder in July 2020 and has since shown to cause harm and is of public health concern.

Methods: Seizures from the Auckland airport and mail centre are analysed by QuickProbe Gas Chromatography - Mass Spectrometry (GCMS) and/or GCMS with comparison to a library database. Seized drugs and drugs from drug checking organisations are analysed by GCMS at ESR also with comparison to a library database.

Drugs in biological samples are screened by liquid chromatography with time of-flight tandem mass spectrometric detection (LC-TOFMS) and quantified by liquid chromatography with tandem mass spectrometric detection (LC-MSMS). The quantitation of the blood was undertaken by using a modified Quechers method for the extraction, appropriate metonitazene reference material and using deuterated zopiclone as the internal standard. The quantitation was undertaken using a Sciex 550 LC-MSMS with a biphenyl column 50X2.1 millimetres with a 2.7 micrometre particle size. Solvent used is 0.1% formic acid in water and methanol. The calibration curve ranged from 0.0002 to 0.05 milligrams per litre blood. Each case blood was analysed in triplicate.

Results & Discussion: Metonitazene was detected in seven coronial cases in New Zealand between October 2022 and October 2023. The decedents ages ranged from 18 to 53 years (mean: 29) and six were male.
The post-mortem metonitazene blood levels ranged from 0.002 to 0.01 milligrams per litre (mean: 0.004).
Yellow powder or yellow tablets were found associated with five of the seven deaths and each of the powders/tablets contained metonitazene and no other drugs. No drug paraphernalia were found associated with the remaining two deaths. During the timeframe of these deaths, most, but not all, of the seizures detected at our border or by drug checking containing metonitazene were also yellow.

Other drugs and drug paraphernalia were also found associated with the five deceased. The drugs detected included fluorexetamine, bromazolam, mephtetramine (MTTA), methamphetamine, dimethylpentylone, isonitazene/protonitazene, ketamine, MDMA and LSD. Many, but not all, of the drugs found at the scene were also detected in the blood of the deceased. In addition, medicinal drugs were found in the blood of six of the seven cases.

In September 2023 High Alert issued a notification in New Zealand following a death and a number of serious hospitalisations resulting from the use of fake oxycodone pills that contained metonitazene. These tablets had been sold on the dark web advertised as oxycodone tablets and had been presented to drug checking services presumed to be oxycodone. Of note, no oxycodone was detected in the blood of the seven coronial cases where metonitazene was detected.

No metonitazene has been detected in a coronial case or in seizures found crossing our border or in our drug checking in New Zealand from November 2023 to March 2024.

Conclusion: The information gathered that provided evidence to the community of the harm metotinazene was causing in New Zealand was gathered from different organisations throughout New Zealand working together cohesively and in a timely manner. These organisations include ESR, enforcement agencies (Police, Customs), drug testing agencies (KnowYourStuffNZ, Drug Foundation, Needle Exchange), health agencies (hospital emergency departments and first responders) and community services.

Continued Information sharing of drug use and harm in the community is crucial in identifying new and emerging drugs to enable drug harm reduction strategies to be developed.

Chemical analysis of recreational products containing semi-synthetic cannabinoids in Germany

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Background & Aims: There is a continuous demand for legal alternatives to controlled psychoactive substances, including cannabimimetic drugs. Since the first identification of hexahydrocannabinol (HHC) in Europe in May 2022, semi-synthetic cannabinoids (SSC) increased in popularity and variety. In Germany, HHC and its derivatives are not subject to the Narcotics Act (BtMG) or the Act on New Psychoactive Substances (NpSG). Thus, within a short period of time, a variety of products such as hash, vape pens, concentrates, flowers, and edibles have been made available in retail shops and online stores, declaring the main active ingredients HHC and its structural analogs hexahydrocannabinol acetate (HHCO), hexahydrocannabiphorol (HHCP) as well as CBD and tetrahydrocannabidiol (H4CBD). The quality and content of these products is not regulated and differences in the advertised and actual content as well as the presence of impurities, e.g. from synthesis, can be expected. The presented work aimed to characterize SSC products available in Germany to determine the major, minor and trace chemical components.

Methods: Starting in June 2023, regular test-purchases (n=42) were made in online and local stores for six months. The commercially available SSC products were analyzed by GC-EI-MS to screen for the main active ingredients as well as other components. Subsequently, quantitative analysis for HHC was performed using GC-MS/MS and testing for the presence of heavy metals was performed by applying ICP-OES.

Results & Discussion: In 86% of products advertised to contain HHC (HHC-products, n=14), HHC was the main ingredient. HHC concentrates containing phytocannabinoids and beta-caryophyllene point towards the semi-synthetic production from plant-extracted CBD. Surprisingly, minor amounts of tetrahydrocannabiphorol and Δ^9-THC were identified in an HHC-product as the main psychoactive components.

In 90% of H4CBD-products (n=10), the H4CBD was the analytically confirmed main compound. Notably, one vaporizer cartridge contained HHC instead of the advertised H4CBD and HHCO, whereas another sample contained significant amounts of HHCO while lacking a corresponding declaration.

Only one HHCP-product contained mostly HHCP, while in a second, only a small amount of HHCP was combined with HHC as the main constituent. Similar to HHCP, most HHCO products contained only small amounts of the advertised content. Notably, Δ^9-THC was identified in HHCO- and HHCP-products more frequently, potentially to mask the lack of the advertised compounds. Hitherto unidentified components detected in HHCP- and HHCO-products may provide further insight into extraction routes and synthesis routes. Diastereomeric ratios of SSCs widely varied in all products and are likely the result of different synthesis procedures.

Many of the unidentified impurities appear to be structurally related to phytocannabinoids or the SSCs present as main component in the respective sample. The consistent association with certain SSCs indicates that they are very likely synthesis byproducts. Besides triethyl citrate, 2-isopropyl-*N*,2,3-trimethylbutanamide, trioctanoin, neither unexpected or potentially harmful chemicals nor elevated levels of heavy metals were detected during the screening.

Conclusion: The screening of the SSC products revealed widely differing formulations and differences between advertised and actual content. Although in some samples unidentified impurities were present, particularly harmful chemicals or heavy metals were not detected. In 38% of cases, the advertised contents of SSC products were not reliably declared. HHCP and HHCO products were found to be mislabeled very frequently (86%), while H4CBD and HHC samples were more likely to contain the advertised compounds (92%). Wrong declaration might result in unexpectedly strong psychoactive effects and adverse events.

NPS O-7

Hemp-derived or semisynthetic cannabinoids: pharmacology of isomers and how it matters for the harm potential of seized drugs

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Background & Aims: The introduction of the 'Farm Bill' (2018) in the US, differentiating hemp (containing 0.3% Δ9-THC or less, on a dry weight basis) from marijuana, created what is called 'a legal loophole' in the cannabis industry. Recultivation of industrial low-THC hemp created an oversupply of CBD-rich products, which was then used as starting material to diversify the markets by producing 'hemp-compliant' THC-analogs. These analogs include Δ8-THC, THC-homologs, hexahydrocannabinol (HHC) and other semisynthetic cannabinoids, that can be derived by chemical conversion of hemp-products. This study aimed at gaining insight into the potential for abuse of purified forms of phytocannabinoids and semisynthetic cannabinoids, to allow prioritization of legislative efforts and research endeavors for the monitoring of these compounds in control programs.

Methods: The potential for abuse was evaluated for a large panel (n=30) of THC-isomers, -homologs and -analogs, that might possibly be derived with semisynthetic procedures. Therefore, this study focuses on the compounds' potential to activate the CB1 receptor, relative to Δ9-THC, the primary psychoactive compound in cannabis. A cell-based NanoBiT® (Promega) β-arrestin2 recruitment assay was used to assess the intrinsic receptor activation potential of the CB1 receptor, which is the prime receptor responsible for the psychoactive effects of cannabis. In addition, seized drug samples of HHC material (Belgium) were investigated with liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) to assess the relative composition of different diastereomers and the implications they have on biological activity.

Results & Discussion: THC-homologs with longer alkyl chain lengths (THCH, hexyl-tail; THCP, heptyl-tail; THC-C8, octyl-tail), together with THC-analogs HHC and hexahydrocannabiphorol (HHCP) and the THC-isomer exo-THC, were identified as compounds with higher CB1 receptor activity than Δ9-THC, based on either potency (EC50) or efficacy (Emax). Other THC-isomers showed lesser potential for CB1 activation, making them less prone to emerge as semisynthetic cannabinoids on the recreational drug market. In general, the pharmacology of stereoisomers (S versus R) was pronouncedly different, with one isomer showing higher CB1 activity than the other. This showed to be important in seized HHC drug products that showed various relative compositions of 9(S)-HHC (21-47%) and always contained more 9(R)-HHC, which was characterized as the epimer with the highest CB1 activity. Literature reports that different epimeric compositions of HHC can be obtained through varying semisynthetic production routes/conditions. Different batches of HHC material could be distinguished based on the epimeric composition, which was consistent with the labeling of the different HHC-containing recipients. Bioactivity-wise, based on the evaluation of different epimeric mixtures of reference standards of 9(R)- and 9(S)-HHC, a decreased relative abundance of the 9(S)-epimer was empirically shown to lead to an increased potency of the epimeric mixture. Hence, HHC material with increased 9(R)-HHC abundance is potentially more harmful.

Conclusion: Multiple derivatives of THC show higher intrinsic CB1 activation potential than Δ9-THC as the primary psychoactive constituent in cannabis. Some of these have already emerged on recreational drug markets (e.g., HHC, HHCP, THCP), others might be anticipated to share the same fate. Monitoring these semisynthetic cannabinoids is encouraged as the dosing (related to the potency studied here) and the relative composition of stereoisomers is hypothesized to greatly impact the harm potential of these drugs, relative to Δ9-THC products. Additionally, determination of the epimeric composition of drug products could be useful for batch identification.

NPS O-8

Isomeric analysis of hexahydrocannabinol (HHC), hexahydrocannabihexol (HHCH), and hexahydrocannabiphorol (HHCP) in products using LC-MS and supercritical fluid chromatograph (SFC)-QTOF-MS

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Background & Aims: In recent years, analogs of Δ^9-tetrahydrocannabinol (Δ^9-THC) have been widely distributed worldwide through the Internet. Hexahydrocannabinol (HHC), synthesized from THC via reduction, was listed as a Designated Substance under Japan's Pharmaceutical and Medical Device Act in March 2022. However, other hydrogenated cannabinoid analogs with varying alkyl side chain lengths at the C3 position of THC, such as hexahydrocannabihexol (HHCH) and hexahydrocannabiphorol (HHCP), have emerged. Several emergency cases were reported in October 2023 owing to the ingestion of gummies containing HHCH in Japan. These compounds possess three chiral carbons and are usually detected in products as mixtures of 9(R)- and 9(S)-epimers. This study evaluated the

content ratios of 9(R)- and 9(S)-epimers of HHC, HHCH, and HHCP in products obtained via the internet through LC-MS analysis. Additionally, chiral analysis of these compounds in the products was carried out using supercritical fluid chromatograph (SFC)-QTOF-MS with a chiral column, and the steric structures were discussed in terms of synthetic raw materials and methods.

Methods: Mixtures of the 9(R)- and 9(S)-epimers of HHC, HHCH and HHCP were synthetized by catalytic hydrogenation of Δ^8-THC, Δ^8-THCH, Δ^9-THCH and Δ^8-THCP using palladium carbon, respectively. Each epimer was purified from the resulting mixtures and confirmed for structure and purity through NMR and LC-MS analyses. Twenty-eight oil-like products (8 containing HHC, 10 containing HHCH, and 10 containing HHCP) obtained via the Internet in 2022 and 2023 were used for the analysis. For LC-MS analysis, acetonitrile was added to the products to achieve a 1 mg/mL concentration, followed by sonication for 5 min and filtration. The extracts were also investigated for chiral analysis using SFC-QTOF-MS. LC-MS coupled with a UV detector was performed under a gradient condition of 0.1% formic acid and 0.1% formic acid/acetonitrile using an ACQUITY UPLC column BEH C18 (2.1 mm i.d. × 100 mm, 1.7 µm, Waters). The content ratio of 9(R)-/9(S)-epimers (R/S ratio) was calculated based on the peak area ratio at UV 280 nm. SFC-QTOF-MS analysis was performed under a gradient condition of supercritical CO_2 and modifier (10 mM ammonium acetate solution in ethanol, isopropanol, and acetonitrile in a 1:1:4 ratio) using a chiral column Trefoil CEL2 (3.0 × 150 mm, 2.5 µm, Waters).

Results & Discussion: In the LC-MS analysis, the R/S ratio of HHC epimers synthesized by reducing Δ^8-THC was 0.7, while the R/S ratio of HHCH obtained from Δ^8-THCH was 1.0, and that of HHCP obtained from Δ^8-THCP was 0.6 under the synthetic conditions employed in this study. On the other hand, the R/S ratio of HHCH synthesized by reducing Δ^9-THCH was 0.2, indicating a prevalence of the 9(S)-epimer compared to HHCH derived from Δ^8-THCH, with a fivefold difference. Across all 28 products analyzed, both 9(R)- and 9(S)-hydrogenated THC analogs were present, albeit in varying quantity ratios. Among the 8 products containing HHC, the R/S ratio was 0.9 -1.6, demonstrating nearly equal proportions of 9(R)- and 9(S)-epimers. In contrast, among the 10 products containing HHCH, the ratio was 0.8 - 9.4, with a tendency towards more 9(R)-epimers. The range was 0.4 - 5.0 for the 10 HHCP-containing products, indicating significant variation between products. In the SFC-QTOF-MS analysis using the chiral column, both 9(R)- and 9(S)-HHCH were detected as racemates in five out of the ten products where HHCH was present. However, enantiomers of the 9(R)- and 9(S)-epimers were not detected in any of the HHC and HHCP products. The racemic compounds detected might have been synthesized through the condensation of the readily available racemate of citronellal with 5-hexylcyclohexane-1,3-dione. Several potential routes for the synthesis of hydrogenated THC analogs exist. For further discussion of the raw materials and synthesis methods of these compounds, it is necessary to investigate the by-products detected in the products.

Conclusion: Variations in the content ratios of 9(R)- and 9(S)-epimers and the presence of enantiomers in these products are likely attributed to differences in raw materials and synthetic methods. The results of this study will provide valuable insights into the profiling of products distributed via the internet. In Japan, analogs of Δ^8- and Δ^9-THC containing alkyl side chains from C3 to C8 (excluding C5), as well as hydrogenated THC analogs with alkyl side chains ranging from C3 to C8, were comprehensively listed as Designated Substances under the Pharmaceutical and Medical Device Act in September 2023 and January 2024, respectively. However, even after they have been regulated, novel THC analogs like acetoxy, methoxy, and hydroxy derivatives have been detected in various products, including food. The distribution of such food products raises concerns regarding potential health hazards from the accidental ingestion of large quantities of the food products, especially among children. Continuous monitoring of products containing these cannabinoids remains imperative.

Another new addition to the SCRA landscape: *in vitro* characterization of the functional activity and metabolites of CHO-4'Me-5'Br-FUBOXPYRA

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Background & Aims: CHO-4'Me-5'Br-FUBOXPYRA or CH-FUBBMPDORA has been one of the latest additions to the recreational drug market, bypassing the Chinese generic ban (2021) on synthetic cannabinoid receptor agonists (SCRAs) due to its new brominated and methylated oxopyridyl core. The substance has been detected and seized in the United States and several countries in Europe. To date, its pharmacological properties remain undefined. Interestingly, structurally similar oxopyridine carboxamides with cannabinoid activity have been described in literature before, which may have inspired the synthesis of the newly emerging SCRA. Furthermore, CHO-4'Me-5'Br-FUBOXPYRA has not been found in biological samples, however it's unclear whether this is the result of low prevalence or difficulty to detect the compound due to the generation of unknown metabolites and potential ab-

sence of the parent compound. This study describes the pharmacological characterization of this new substance, a structural analog found in literature, and a seized powder confirmed to contain CHO-4'Me-5'Br-FUBOXPYRA, and investigates its metabolization profile.

Methods: The potency and efficacy of the substance, an analog and a seized powder were evaluated by means of CB_1 and CB_2 βarr2 recruitment assays, based on functional complementation of a split nanoluciferase enzyme and the measurement of bioluminescence. Metabolite studies were conducted via human liver microsome (HLM) incubation and metabolites were identified using liquid chromatography coupled to a quadrupole time-of-flight mass spectrometer (LC-QTOF-MS).

Results & Discussion: CHO-4'Me-5'Br-FUBOXPYRA showed a limited activation potential at both cannabinoid receptors. At CB_1, we found a potency (EC_{50}) of 3782 nM and an efficacy of 21.8 %, relative to the E_{max} of the reference SCRA CP55,940. At CB_2, CHO-4'Me-5'Br-FUBOXPYRA had a potency of 4267 nM and a relative efficacy of 17.0 %. The seized powder exhibited a pronounced increase in CB_1 activity with a maximal receptor of 351% (compared to CP55,940) at the highest tested concentration of 50 µM. No accurate EC_{50} value could be calculated as no plateau in receptor activation was reached. These results are suggestive of the presence of a contaminant with higher CB_1 activation potential (although analytical characterization had not revealed another compound). At CB_2, the powder showed a similar but slightly higher activation potential as the reference standard, with an EC_{50} of 6079 nM and a relative E_{max} of 45.3%. Comparison with the analog described in literature, which carries a heptyl head group instead of a hexyl moiety, revealed a very similar activation profile at both receptors. *In vitro* incubation with HLMs led to the identification of four metabolites of CHO-4'Me-5'Br-FUBOXPYRA, which were all the result of an extensive oxidation of the parent molecule, followed by hydroxylation of the cyclohexyl head group (3 metabolites) and of the methyl function on the core (1 metabolite).

Conclusion: Given the dynamic nature of the SCRA market, characterization of newly emerging compounds remains key to adequately inform health care providers and law enforcement agencies and to timely act to combat the surge of more new substances. Overall, the newly emerged CHO-4'Me-5'Br-FUBOXPYRA showed a rather weak to moderate cannabinoid activation potential, similar to its structural analog previously reported in literature. On the other hand, different preparations may exhibit a much greater cannabinoid activity, as exemplified by the investigated seized powder. This may potentially lead to more pronounced cannabinoid-related toxicity in users. In addition, metabolite identification may facilitate the detection of the substance, allowing for a more accurate monitoring of the use of this new SCRA.

Scientific Session 3 – New analytical approaches

14:00 – 16:00 Tuesday, 3rd September, 2024

Chair: Christophe Stove, Andrea Steuer

NAA O-1

Development of a multi-omics sample preparation workflow for comprehensive Metabolomics, Lipidomics and Proteomics datasets using a single tissue sample

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Background & Aims: In recent years, various new analytical approaches emerged that investigate the potential of utilizing endogenous molecules to solve forensic toxicological challenges. This includes the search for biomarkers for the indirect detection of xenobiotics/xenobiotic classes/new substances, longer windows of detection and postmortem time-dependent concentration changes to name a few. Particularly metabolomics (small metabolites), lipidomics (lipids) and most recently proteomics (peptides/proteins) approaches have been used. A frequent problem is a very specialized sample preparation for the three different compound classes. Metabolomics/lipidomics workflows often differ significantly from proteomics sample preparation approaches. In cases where a comprehensive study of all endogenous molecules is intended, in the worst case, three different samples are needed for three specialized extractions. This leads to issues with the comparability between samples (e.g. tissue homogeneity, additional freeze-thaw cycles) and difficulties combining the multi-omics results for a biological interpretation. Specifically in human time-dependent postmortem investigations, with repeated sample collections, a limited sample amount (e.g. using biopsy samples) can pose additional problems. The aim of the current study was to develop a multi-omics sample preparation workflow using a small single postmortem tissue sample that aids

in comprehensive metabolomics, lipidomics and proteomics datasets. The focus was on parameters for optimal sample homogenization and extraction across all -omics approaches.

Methods: Human postmortem muscle and liver tissue samples (ethics approval ETH18-2999) were used to evaluate different homogenization parameters (homogenization solvents (n=5), pulsing frequencies (n=5), sample material-to-homogenization solvent ratios (n=4) and sample amounts (n=4)) and extraction parameters (extraction solvents/mixtures (n=10)). The general sample preparation workflow included bead homogenization (3 mm zirconium beads), followed by extraction and compound class specific analysis with liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based analytical methods as well as 1D-SDS polyacrylamide gel electrophoresis (PAGE) and a bicinchoninic acid (BCA) protein assay. Suitability of homogenization parameters were assessed based on a targeted evaluation of the Metabolomics data (microflow-LC-ion mobility separated qTOF analysis, data independent acquisition; peak intensities and reproducibility of technical replicates) and based on the numbers/intensities of protein bands visible on the 1D SDS-PAGE gel (run on the protein fraction). The suitability of the extraction solvents was assessed and ranked based on the following parameters: targeted and untargeted (number of features, reproducibility of technical replicates, compound classes) evaluation of the Metabolomics and Lipidomics dataset (high flow-LC-orbitrap analysis, data dependent acquisition), untargeted bottom-up Proteomics data (microflow-LC-ion mobility separated qTOF analysis; identification of peptides/proteins from the human proteome and reproducibility between technical replicates), 1D SDS-PAGE (gels run on the protein- and metabolite fraction of the extracts) and BCA assay results (protein quantification). A variety of software solutions were used for data processing, including, Skyline (V23.1), MS-Dial (V4.9), Sirius (V5.8) and Progenesis QI for proteomics. Statistical evaluation was conducted in R (V4.3).

Results & Discussion: A methanol-to-water mixture (2:1, *v/v*) added in a 1:10 sample material-to-homogenization solvent ratio was found to lead to the best Metabolomics and visual protein results. A 20 mg tissue sample was found to be sufficient to result in comprehensive datasets, not significantly improved by the use of 40 mg tissue. The use of such a small sample amount is ideal for studies with access to only limited sample amounts. Several pulsing frequencies were trialed. For practicality and to prevent sample degradation due to heat, 3x30 s was chosen with a 1 min pause on ice in between pulses. The comparison of the efficiency of different extraction solvents was conducted on both 1- and 2-phase solvent mixtures. Commonly used extraction solvents for Proteomics studies (e.g. methyl tert-butyl ether:water or dichloromethane:water mixtures), lead to significantly weaker or less reproducible Metabolomics and Lipidomics results, compared to 1-phasic extraction solvents more commonly seen in small molecule studies. A simple protein precipitation of the homogenized tissue samples with a methanol:acetone mixture (9:1, *v/v*), overall, showed the best compromise with achieving comprehensive Metabolomics, Lipidomics and Proteomics datasets.

Conclusion: In summary, a multi-omics sample preparation workflow was established that can lead to comprehensive targeted and untargeted Metabolomics, Lipidomics and Proteomics datasets from a single tissue sample (20 mg), fitting into existing Metabolomics workflows. Optimal homogenization and extraction solvent parameters were suggested with special emphasis on postmortem tissue samples. While this method can serve as a basis for multi-omics studies in other matrices, the results would need to be confirmed. To counteract issues with the reproducibility of Metabolomics/Lipidomics technical replicates based on the pure organic nature of the extracts, it is recommended to use a separate vial for each technical replicate/injection (risk of evaporation through pierced lids). The proposed multi-omics method has the great potential to expand future -omics studies in forensic toxicology, leading to comprehensive Metabolomics, Lipidomics and Proteomics datasets using a single sample. Additionally, it seems feasible to use the method for the detection of xenobiotics, potentially opening the door for -omics results from routine case samples.

Acoustic ejection tandem mass spectrometry for high-throughput screening of phencyclidine-type substances in urine, including authentic cases

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Background & Aims: Phencyclidine-type substances have fluctuated in the drug abuse market, from PCP to ketamine, and the abuse of phencyclidine-type substances has undoubtedly posed a great challenge to anti-drug efforts around the world. Retrospective analysis can be used to analyze the past situation to determine the current or future situation, and to target monitoring or control. However, the huge sample size would be time-consuming. Therefore, in this study, we will construct a rapid screening method for 9 common phencyclidine-type substances based on urine relying on Echo-MS/MS, and the accuracy of the rapid screening method will be examined by a validated UHPLC-MS/MS method.

Methods: **Target selection.** Based on previous research on the abuse of phencyclidine analogs, we selected 9 substances that have appeared in prosecutions as targets: 2-FDCK, tiletamine, O-PCE, ketamine, DCK, nor2-FDCK, 2-BrDCK, 2-F-2-oxo-PCE, and norDCK.

Sample preparation. The urine was centrifuged for 5 min, and the supernatant was diluted 20-fold with water and pipetted into a 384-well plate, which was then covered with a dust film. The plate was centrifuged for 5 min and then shaken to obtain a flat concave surface.

Echo-MS/MS. The Echo-MS/MS system was a combination of an Echo® MS and API 6500+ TRAP triple quadruple linear ion trap mass spectrometer. A 20 nL volume was ejected and transported by the carrier solution (acetonitrile/water, 70:30, v/v) into the mass spectrometer at a flow rate of 0.4 mL/min. The mass spectrometer was operated in the positive electrospray ionization and MRM modes.

Method validation. Selectivity was investigated by testing different blank urine specimens from many drug-free donors. A series of decreasing concentrations of drug-spiked urine was prepared (10, 5, 2.5, 1.25, 0.625, and 0.3125 ng/mL), and evaluated to determine the LOD. The LOD is the concentration at which the peak of the measured substance is not interfered with, and the relative standard deviations of 5 consecutive ejection results is < 15%.

UHPLC-MS/MS. Sample elution (5 µL injected) was performed at a flow rate of 0.3 mL/min with mobile phase A (water, 0.1% formic acid, 5% acetonitrile, 20 mmol/L ammonium acetate) and mobile phase B (acetonitrile) on a Phenomenex Kinetex® Biphenyl 100Å LC column. After diluting (900 µL methanol added to 100 µL urine), a 200 µL aliquot of the supernatants was transferred into a vial for UHPLC-MS/MS analysis.

Results & Discussion: **Sample preparation.** For Echo-MS/MS, urine was processed by simple dilution to accommodate "fast" requirements. Since the maximum sample loading volume was only 60 µL and a higher the proportion of organic phase in the dilution accelerates the evaporation rate, only water was used here as the dilution solvent.

Method validation.

Echo-MS/MS. By analyzing 40 blank urines using Echo-MS/MS, the peak area was determined with a confidence level of 99.85%. Above this area, it can be determined as detected. Due to interference causing unforeseeable deviations in the concentration of the analyte, we added standards to blank urines from different sources to determine their concentration deviations. When the RSD is less than 15%, we believe that interference has no effect on detection at this concentration. Based on the two methods mentioned above, we ultimately chose 10 ng/mL as the LOD and analyzed 250 samples simultaneously using Echo-MS/MS and UHPLC-MS/MS. Echo-MS/MS has extremely high accuracy in sample screening: when the detection limit of Echo-MS/MS is above 10 ng/mL, the detection accuracy of Echo-MS/MS is 100%. In the test, 250 specimens were analyzed in 1.5 h.

UHPLC-MS/MS. The quantitative method established based on UHPLC-MS/MS met the validation requirements in all aspects. The LOD and LOQ values for all analytes ranged from 0.1–1 ng/mL and 0.5–2 ng/mL, respectively.

Positive specimens. After analysis by Echo-MS/MS, 4 of the 250 urine samples were definitely detected as containing drugs, and 1 was suspected to contain them. Subsequent validation and quantification by UHPLC-MS/MS demonstrated that 7 phencyclidine-type substances were indeed detected: 2-FDCK (25,550 ng/mL), tiletamine (2,430 ng/mL), O-PCE (59,290 ng/mL), ketamine (220 ng/mL), DCK (2 and 164 ng/mL), nor2-FDCK (9–2,965 ng/mL), and 2-F-2-oxo-PCE (7–1,393,000 ng/mL). Notably, multiple substances were detected in each of the 5 urine specimens, suggesting the possibility of cross-abuse of phencyclidine-type substances with similar drugs.

Conclusion: This was the first fast screening method for phencyclidine-type substances based on Echo-MS/MS, which greatly reduces the analytical time, and can accomplish in 1.5 h what UHPLC-MS/MS needs 3 days to complete. And the samples can be analyzed without complicated sample preparation, and also can obtain good detectability.

NAA O-3

A new alternative for dried blood spots papers: nanofibrous sorbents

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Background & Aims: Nanofiber production is important for the production of functional materials. The electrospinning method used in producing nanofibers is effective in terms of ease of application and the richness of polymers.

This method is that the fibers produced with this technique have nano-sized diameters and the polymers used can be used both as melt and spinning solutions. This study, nanofibrous sorbents were synthesized by electrospinning method and used as paper/sorbent in the Dried Blood Spot(DBS) for the determination of 11-nor-9-carboxy-THC(Δ9-THC-COOH),amphetamine,3,4-methylenedioxy-N-methylamphetamine(MDMA),morphine and benzoylecgonine. Synthesized nanofibrous and Whatman903 which dries slowly and has a risk of contamination during the study, were statistically compared using blood.

Methods: All standards and internal standards detection conditions were optimized by LC-MS/MS. One-mL methanol-acetonitrile40:60(v/v) was added to a 50-µL blood and liquid-liquid extraction(LLE) was performed. The organic phase was evaporated under nitrogen. The residues were dissolved in 150-µL of mobile phase mixture and vials were injected into LC-MS/MS. DBS(Whatman903) samples were dried at room temperature for 2-h after dripping 50-µL of blood, and the dried spots were cut and transferred into 1-mL of methanol-acetonitrile40:60(v/v). After 30-min of ultrasonic bath, paper/sorbent was removed from the solvent and the procedure for blood sample was applied. Analytical method validation was performed using two different matrices for whole blood and DBS (Whatman903). The method validations were performed on parameters of specificity, accuracy, the lower limit of determination(LOD), the lower limit of detection(LOQ), linearity, precision, stability, and carryover.

Cellulose acetate(CA) and thermoplastic polyurethane(TPU) membranes were produced by electrospinning. Electrospinning of polymer solutions was carried out by using NS Plus Nanospinner Electrospinning. Nanofiber synthesis experiments were carried out at different voltages and polymer solution feeding rates. Electrospinning of each membrane was carried out 24 and 48 hours to produce membranes with two different thicknesses, and a double-face membrane was produced consisting of CA and TPU nanofibres on each side, which was denoted as a "mix mode". A deacetylation process was applied to increase the durability of the membranes including CA. The morphology of the produced nanofibers was analyzed by scanning electron microscope and Fourier-transform infrared spectroscopy(FT-IR). The water retention capacity of the samples was tested. 30 real samples positive for Δ9-THC-COOH,amphetamine,MDMA,morphine, and benzoylecgonine were analyzed with the validated DBS method(Whatman903). With the same validated method, the same real samples were analyzed with five synthesized nanofibers (24-hour CA,48-hour CA,24-hour TPU,48-hour TPU, and mix mode), and the results were statistically analyzed.

Results & Discussion: LLE was carried out at different pHs of water, methanol, isopropyl alcohol, ethyl acetate, diethyl ether, hexane, acetonitrile, chloroform, and their mixtures in working whole blood and DBS. While the most compatible results were obtained at the methanol-acetonitrile ratio of 40:60(v/v). For whole blood and DBS methods, each analyte can be distinguished within the sample it contains, LODs were between 2-7.5ng/mL; LOQ between 10-25ng/mL. All correlation coefficients exceeded 0.995(linear to LOQs-500ng/mL). Analytical recovery ranged from 84.9-113.5% of the expected concentration for both intra-day and inter-day. Stability was ensured under storage conditions of 24 hours at -20°C and freeze-thaw cycles for 10 and 30 days.

For CA and TPU fiber diameters of 358.60nm and 233.90nm, respectively were chosen due to lower nanofiber diameter and smooth nanofiber morphology. FT-IR spectra show after deacetylation, it was observed that characteristic peaks of acetyl groups disappeared. While the water retention capacity of Whatman903 in paper structure is approximately 230% by weight, it was determined that cellulose-based nanofibers are in the range of 280-320% by weight, depending on the thickness. In the comparison study, correlation test results were evaluated for the results of samples (n=53) positive for THC-COOH, amphetamine, MDMA, morphine, and benzoylecgonine. Correlation coefficients were calculated as Whatman903:CA-24h 0.941, Whatman903:CA-48h 0.982, Whatman903:TPU-24h 0.881, Whatman903:TPU-48h 0.951, Whatman903:Mix 0.973. When the results of Whatman903 filter paper and nanofibers were compared, cellulose and mixed nanofibers produced in 48–h can be used interchangeably.

Conclusion: In this study, a sensitive, selective, and repeatable LC-MS/MS method was developed for the quantitative analysis of illicit substances. This study was designed to overcome the limitations of the DBS technique and to obtain more efficient, sensitive, and accurate results by producing alternative new materials, the most common method used in nanofiber production was the electrofiber spinning method. Nanofibrous surfaces have very thin fiber diameters of nanometer dimensions and therefore very high surface area and porosity. Thus, nanofibers, especially those produced from hydrophilic polymers or subsequently made hydrophilic, have high liquid absorption capacity. Since nanofibrous sorbents have a higher liquid absorption capacity, they trap the liquid they absorb within the sorbent and reduce the problem of contamination in the environment to zero. This study was supported by TÜBİTAK-1002(Project No:211S955). This study was deemed worthy of the Anya Pierce Young Scientist award by EWDTS referees.

NAA O-4

Comprehensive characterization of biological fluids from subjects tested positive for illicit drugs using supercritical fluid chromatography mass spectrometry with orthogonal ionization and fragmentation techniques

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Background & Aims: Exogenous compounds such as drugs, pollutants, bacterial toxins and related metabolites exert various effects on biological systems, leading to alterations of endogenous metabolic pathways. Xenobiotic elimination from the body primarily occurs through hepatic elimination. Excess and continuous exposure stress the liver, potentially causing serious organ damage and impairing its predominant role in lipid metabolism. Evaluating the exposome provides valuable insights into subjects' health, lifestyle choices and allows to group subjects based on the frequency and duration of exposure to specific substances.

Frequently untargeted liquid chromatography electrospray ionization mass spectrometry collision-induced dissociation (LC-ESI-MS-CID) techniques are used to evaluate xenobiotic-induced metabolic alterations but are challenged by unambiguous compound identification (ID). Confidence in analyte ID can be increased through orthogonal ionization and fragmentation techniques, such as electron ionization (EI), atmospheric pressure photoionization (APPI), or electron-activated dissociation (EAD). While EI generates information rich fragmentation spectra it is restricted to gas chromatography and thereby limited to small thermostable molecules. Contrary to that, ESI and APPI are considered as soft-ionization techniques which can be used for a wider range of compounds using liquid chromatography. Compared to ESI, APPI does allow radical cation formation with CID spectra usable for EI library searches with 300,000+ molecules and is superior for nonpolar analytes (Mueller et al., 2022). The fact that both can be coupled to liquid chromatography allows to perform cross validation of features with similar retention times using EI-library searches and CID MS/MS library searches.

Within this work we apply untargeted data-independent SWATH workflows using ESI (Klont et al., 2020) with supercritical fluid chromatography, ESI-EAD and APPI-SWATH of radical cations for xenobiotic, metabolomic and lipidomic screening. The multimodal approach was applied for the analysis of plasma and urine from subjects tested positive for illicit drugs, while also investigating xenobiotic-associated alterations of biomolecules.

Methods: Urine samples were diluted 1:1 in methanol and plasma samples were extracted using MTBE/methanol/water (10:3:2.5). General screening was performed using ESI-SWATH and APPI-SWATH on a TripleTOF 6600 (Sciex). The polar fraction was separated on a diol column and the nonpolar fraction using a C18 column with supercritical fluid chromatography (SFC) on a Nexera UC system (Shimadzu). Multivariate analysis was used to discover features which define subject groups. Features of interest were identified by MS/MS library searches for ESI and EI library searches for APPI. Putative molecules were validated using analytical standards. Lipids of interest were reanalyzed using ESI-MRM-HR-EAD on a ZenoTOF 7600 (Sciex) and APPI-SRM-EPI on a QTRAP 6500. De-novo structural elucidation of lipids was performed using R. All APPI experiments were performed using chlorobenzene as dopant.

Results & Discussion: Untargeted SFC-ESI-SWATH-MS screening allowed to identify illicit drugs, related compounds including metabolites, tobacco, and cutting agents, alongside lifestyle-related compounds such as painkillers. Additionally, metabolic and lipidomic alterations associated with illicit drug were observed. The additional use of SFC-APPI-SWATH-MS with NIST EI library searches allowed cross validation of compounds with similar retention times obtained from SFC-ESI. The orthogonal ionization efficiency of APPI towards ESI increased the amount of putative compounds identified such as benzo[a]pyrene, a compound with elevated plasma concentration in smokers. Alike, conducting NIST EI Library searches allows for the assignment of more putative compounds, due to a tenfold greater number of molecule entries compared to MS/MS libraries. Multivariate statistical analysis showed larger differences for APPI-CID than for ESI-CID. This can be attributed to the generation of more information-rich EI-like spectra generated through the collision-induced dissociation of radical cations. This has proven particularly useful for steroids which show low ionization efficiency with uninformative fragmentation spectra of $[M+H-H_2O]^+$ when using ESI, but generate dominant radical cations using APPI. De-novo structural elucidation of lipids, without the need of libraries ,was performed using SFC-ESI-EAD and SFC-APPI-CID. SFC-APPI-CID was superior for lipid classes which do not ionize well in (+)-ESI, such as fatty acids, cholesteryl esters, mono-di and triglycerides, and MRM-HR with EAD for the remaining lipid classes. Last, the use of SFC-ESI-CID, SFC-ESI-EAD and SFC-APPI-CID of radical cations allowed validation of putative compounds by comparing their retention times and three orthogonal fragmentation spectra with those of analytical standards.

Conclusion: Integrating typical ESI-SWATH workflows with ESI-EAD and APPI-SWATH of radical cations increases the amount of putative compounds identified, which is attributed to their complementary in ionization and fragmentation. In particular, the CID fragmentation of radical cations generates spectra which can be used for structural informative EI library searches. Altogether, the described multimodal approach improves screening and characterization of exogenous and endogenous compounds related to illicit drugs in urine and plasma.

Drug characterization and impurity mapping using R

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Background & Aims: Illicit drug markets continue to experience significant growth, and while there is a continuous need to determine the main pharmacologically active substances, the detection and characterisation of minor compounds is starting to receive significant interest for providing strategic, tactical and public health intelligence.

Limited research has been conducted to examine the presence of inactive compounds or other substances in illicit drug samples. These chemicals, known as adulterants or cutting agents, can be impurities which arise from different processes during the drug's manufacturing, packaging, transportation etc. As they are typically present in small concentrations, their observation in analytical results is a challenge and requires significant manual effort.

This study looks at the use of an automated approach to investigate the presence of minority substances in illicit drugs using an R script. R as a programming language is receiving increasing attention within the scientific community to speed up analysis, but it has not yet been applied to this area of research. The aim of this work is to directly analyse gas chromatography -mass spectrometry (GC-MS) data to systematically detect and assign minority substances in all processed samples.

Methods: Samples included in this study were part of an ongoing non-judicial seizures drug monitoring project. These samples were analysed by GC-MS in full scan mode, and the output data were converted in a format suitable for long term preservation. The data are processed in R to (i) detect the presence of minor peaks and (ii) compare them to already known substances present in a self-built reference library. The R script compares mass spectra between the peaks of interest and the ones available in the library using vectors and indexing similarity with spectral contrast angle. This is simply calculated using the relative intensities of product-ion peaks at the m/z values.

Results & Discussion: 107 samples were analysed by GC-MS. With the developed R code set to extract peaks that are three times the signal-to-noise ratio of the baseline, using the total ion chromatogram, a total of 3,825 peaks of interest were detected throughout the entire series. Among the 3,825 peaks identified, 119 were classified as distinct due to their varying retention times. Analysis of the data reveals that these 119 peaks can be entirely accounted for by examining only 27 out of the 107 samples. Additionally, by selecting only 10 samples, 102 out of the 119 peaks can be identified.

Peak assignment was carried out by comparing peaks between samples using the MS data. The comparison and matching of each detected peak were done by the measure of similarity (and the use of angular vectors), which returns a matching factor. A matching factor of 0 indicates an opposite match, while 1 denotes a perfect match to the peak used for comparison. These peaks used for comparison may not be attributed to a specific compound, as the "reference peak" could be sourced from another sample rather than a compound reference library. In this way, additional analysis can be carried out using the matching factors and the individual peak retention time to establish groups and relationships between samples, thereby facilitating the subsequent identification of compounds.

Conclusion: The research demonstrates the feasibility of developing a facile method to systematically detect chemicals present in a large series of samples. With careful consideration, a list of peaks of interest present in one or more samples can be established by using a targeted number of samples. Such an approach is of great interest for facilitating and expediting data analysis, as well as determining commonalities between samples. The outcomes of this approach could significantly enhance forensic intelligence in drug profiling for investigation and operational work, providing additional information to aid in identification, including geographical sourcing as well as establishing possible relationships between batches of seized drugs.

Development of a green analytical toxicology method based on supramolecular solvent (SUPRAS) microextraction for the analysis of benzodiazepines in vitreous humor

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Background & Aims: The toxicological analysis of *post-mortem* samples in forensic casework is essential to investigate the presence or absence of drugs in cases of suspected intoxication. The vitreous humor (VH), a gelatinous fluid that fills the posterior segment of the eye, has stood out as an alternative biological matrix in forensic in-

vestigations due to the existence of few interfering compounds for the analytical process and its stability. In this context, the analysis of psychoactive substances like benzodiazepines (BZs) and their metabolites in VH is crucial for determining the cause of death and assessing the extent of the abuse issue. BZs are one of the most prescribed drugs used as anticonvulsant, anxiolytic, muscle relaxant, and sedative-hypnotic agents.

Faced with challenges in forensic drugs analysis, the approach of supramolecular solvent (SUPRAS) microextraction could be highlighted as a Green Analytical Toxicology (GAT) procedure and it offer intrinsic advantages, mainly related to the extraction efficiency in diverse analytes. The SUPRAS microextraction occurs with the combination of the salt (sodium sulfate) and the alcohol (1-hexanol) that promotes the extraction of the sample with the formation of two phases, a continuous phase, and a dispersed phase, generated from the association of different molecules in a self-organizing system, being able to extract the BZs from the VH. The objective of this work was to develop and to validate a SUPRAS microextraction method to identify BZs in VH.

Methods: Initially, a Plackett-Burman (PB) experimental design was conducted, for parameters as sodium sulfate amount, agitation time, alcohol volume, and matrix volume. The absolute area of diazepam at different conditions through analysis by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) were evaluated and the best conditions were selected. Thus, a rotational composite central design (RCCD) was set up, with two replicates and five central points (2^3). The parameters were agitation time, alcohol volume, and matrix volume. Chromatographic separation was performed with a Raptor Biphenyl (2.7 µm, 100 x 2.1 mm, Restek, Bellefonte, PA, EUA) column in a LCMS8045 (Shimadzu®, Kyoto Japan) operating in gradient mode with mobile phase A (Water) and B (Methanol), both containing 2 mM ammonium formate + 0.1% formic acid. The mass spectrometer was equipped with an electrospray ionization source, operating in negative mode. The total run time of analysis was 8 minutes.

The sample preparation consists with adding the aliquot of 300 µL of VH to a 2 mL polypropylene tube containing 20 mg of sodium sulfate and the addition of 20 µL of diazepam-d5 2000 ng/mL (internal standard). The tube is vortexed for 10 seconds. Then, 200 µL of 1-hexanol is added, homogenized for 7 minutes at 2500 RPM, centrifuged for 5 minutes at 12500 RPM, and 100 µL of the supernatant is transferred to another tube. Subsequently, the solution is dried under nitrogen flow at 40°C and resuspended with 100 µL of water. Finally, 2 µL is injected into the LCMS8045.

Results & Discussion: The following BZs and metabolites were selected: Lorazepam, oxazepam, nitrazepam, clonazepam, nordiazepam, flunitrazepam, temazepam, alprazolam, diazepam, 7-aminoclonazepam, flurazepam and midazolam. A calibration curve was determined from 10 to 1000 ng/mL and from 1 to 100 ng/mL for 7-aminoclonazepam (linear regression, $1/x^2$ weighting, $r^2 \geq 0.99$). Quality controls (QCs) were determined at 30, 400 and 800 ng/mL as Low-Quality Control (LQC), Medium-Quality Control (MQC) and High-Quality Control (HQC), respectively and they did not vary more than 20% from the nominal concentration after preliminary experiments.

Conclusion: A GAT method was developed using SUPRAS microextraction for the analysis of BZs in VH. The method will be validated as recommended by the AAFS Standard Practices for Method Validation in Forensic Toxicology guidelines, and parameters as bias and precision, carryover, interference studies will be evaluated in different days of work. After that, the method will be applied for the analysis of authentic *post-mortem* VH samples.

NAA O-7

Activity-based characterization of opiates, opioids and NPS molecules, including their metabolites: a forgotten technology with new forensic applications

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Background & Aims: Fentanyl and its structural analogs sufentanyl and alfentanyl are potent analgesics of the 4-anilidopiperidine class of opioids, which are clinically used in the management of pain. However, some fentanyl analogs, such as para-fluorofentanyl, are known as designer drugs (New Psychoactive Substances, NPS) and have been encountered in illicit drug traffic. Several of the illicit fentanyl homologs encountered to date are believed to have potencies much higher than fentanyl itself (e.g. carfentanyl). Knowledge of their pharmacology is often limited to their analgesic potencies as determined by *in vivo* tests. These results provide little pharmacological evidence with respect to their affinity and selectivity for mu, kappa, and delta opioid receptors, through which these ligands mediate their actions leading to respiratory depression, and often in a recreational setting resulting in coma and death.

The aims of our study are i) a functional, activity-based characterization of opiates, opioids and their metabolites, including the latest synthetic opioids such as the nitazenes, known as New Psychoactive Substances (NPS); ii) a site-directed mutagenesis investigation to reveal the role of important amino acids involved in opioid type selectivity (mu, kappa and delta) for ligands such as fentanyl and NPS molecules, including their metabolites.

Methods: The pharmacological profile was investigated of a number of fentanyl analogs on wild-type and mutated human mu, kappa, and delta opioid receptors, co-expressed with GIRK1/GIRK2 potassium channels and RGS4 proteins in a host cell type, called *Xenopus laevis* oocytes. We have applied the 2-microelectrode voltage-clamp technique in order to measure potassium currents as readout, following activation of the opioid receptor (mu, kappa or delta; wild-type or mutant), coupled to inward rectifier GIRK1/GIRK2 potassium channels via recruitment of endogenous G proteins of the host cell. We investigated if mutation of residues Trp-318 and His-319 in the mu opioid receptor to their corresponding residues in kappa and delta opioid receptors provides the molecular basis for mu/delta selectivity and mu/kappa selectivity.

Results & Discussion: We demonstrate that para-fluorofentanyl more potently activates GIRK1/GIRK2 channels through opioid receptors than fentanyl and that the p-fluoro substitution also changes the potency profile from mu > kappa > delta (fentanyl) to mu > delta > kappa (para-fluorofentanyl). Using site-directed mutagenesis, we found that changes in EC50 values for the W318L and W318Y/H319Y mu opioid receptors show a partial contribution of these residues to the decreased GIRK1/GIRK2 channel activation by fentanyl analogs through kappa and delta opioid receptors. The most pronounced effect was observed for para-fluorofentanyl, suggesting that an interaction between the 4-fluorophenylpropanamide moiety of the drug and residues Trp-318 and His-319 is important for the resulting enhanced GIRK1/ GIRK2 channel activation through the mu opioid receptor. We demonstrate that the mutation W318L confers delta-like potency for morphine on the mutant mu opioid receptor. Finally we also show that morphine-6-glucuronide and morphine-3-glucuronide,well known phase 2 metabolites of morphine, are opioid receptor agonists with different potencies and that the potency of morphinan receptor ligands can be changed by selective mutations of the mu opioid receptor at positions Trp-318 and His-319.

Conclusion: We conclude that the heterologous expression in Xenopus laevis oocytes of cloned wild-type and mutant opioid receptors together with ion channels (GIRK1/2) that are recruited upon activation of mu, kappa and delta opioid receptors, and studied with the voltage-clamp technique, is a platform with great potential for an activity-based characterization of opiates, opioids, NPS molecules and their metabolites. With the ever growing number of NPS molecules appearing on the market, in particular the family of the nitazenes, which are very potent synthetic opioids, we believe that the technology reported by us here will help us to elucidate new forensic cases that undoubtedly will occur.

Application of chemometrics in the interpretation of diclazepam and its metabolite ratios

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Background & Aims: Diclazepam is a 'designer' benzodiazepine derivative and modified version of the existing pharmaceutically prescribed benzodiazepine diazepam. There is little knowledge regarding its pharmacokinetics, it has never undergone clinical trials, and it was deemed unfit for human consumption in the early 1970s. However, the possible illicit use of diclazepam as both a recreational and a drink spiking drug has recently been reported. Diclazepam metabolises into three pharmaceutically prescribed benzodiazepines (delorazepam, lormetazepam, and lorazepam) with overlapping metabolic pathways, which could make the identification of the parent drug challenging. It could therefore be justifiable to suspect that diclazepam may simply not have been searched for, overlooked, and not identified with current understandings.

The aim of the research was to investigate whether it is possible to identify delorazepam, lormetazepam, and lorazepam as parent drugs, or metabolites of diclazepam using a chemometric approach.

Methods: Diclazepam metabolises into both delorazepam and lormetazepam simultaneously, and both of these metabolites then proceed to metabolise into the final product, lorazepam. Therefore, for this study, diclazepam, delorazepam, and lormetazepam were metabolised in vitro using pooled human liver microsomes over 240 minutes to establish metabolite ratios of metabolite and parent drug. Liquid-liquid extraction (LLE) was used to extract diclazepam and its metabolites from the incubated microsomes over different incubation times, and analytes detected by a validated gas chromatography-mass spectrometry (GC-MS) method. Principal component analysis was performed to establish the relationships between diclazepam and its metabolites, and to investigate whether the the differentiation of diclazepam, delorazepam, and lormetazepam could be determined based only on the detected quantities of their respective metabolites.

Results & Discussion: The detected diclazepam and its metabolite ratios proved that it is possible to differentiate whether the parent drug was diclazepam, or one of its metabolites. Novel chemometric models using principal component analysis showed the potential for differentiating between parent benzodiazepines. This was initially tested by compiling available literature data for commonly detected benzodiazepine concentrations in biological matrices, and then applying the PCA model based on the recorded metabolite proportions.

In addition, in vitro metabolism revealed information regarding previously unexplored possibilities for diclazepam metabolites behaving in ways that were not expected. The parent drugs metabolised and decreased in concentrations to form their metabolites as expected until 120 minutes of incubation. However, it was suspected that the metabolites then went on to reform back into their parent compounds, either directly or indirectly via intermediate product formation since their concentrations were shown to decrease, while their parent drug concentrations simultaneously increased, a reverse metabolism process that has previously been shown to occur for some steroids and chlorpromazine.

Conclusion: This research has sought to contribute to the gap in knowledge for diclazepam analysis, its metabolism and detection, and to help laboratories become more prepared with results interpretation. The main focus of this research was to see whether one could differentiate between which parent drug had been consumed based on metabolite detection, and this research has confirmed that this is possible.

PCA models were able to differentiate between the incubations of diclazepam, delorazepam, and lormetazepam based only upon diclazepam metabolites being present, and not diclazepam itself. In particular, it was able to differentiate between the three drugs when only two of the metabolites were detected.

Therefore, the findings from this research could contribute to improving and advancing drug identification and interpretation methods in biological samples.

NAA O-9

Exploring electromembrane extraction as a simple and green alternative for the analysis of New Psychoactive Substances

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Background & Aims: New Psychoactive Substances (NPS) continue to appear in the drug market, posing harm to human health and challenging law enforcement. More than 1.200 substances with heterogeneous chemical structures have been reported, stressing the need for sample preparation techniques suited to analyze these emerging drugs in biological samples. Electromembrane extraction (EME) is a miniaturized technique in which charged molecules are extracted from biological specimens through a porous membrane into an acceptor solution. In EME, the use of an electric field as the driving force for mass transfer provides high analyte recovery in short extraction times. Several applications of EME to different groups of substances and biofluids have been described. In this work, we present the application of this technique in a prototype 96-well plate setup for NPS analysis in whole blood. Twenty synthetic cathinones (SC) within a wide log P range (1.18-3.65) were thus chosen as model analytes due to their increasing relevance in the NPS scenario.

Methods: Extractions were performed using 120 µL of blood acidified with 120 µL of 250 mM HCOOH. Only 3 µL of an organic solvent was used and the acceptor phase consisted of 50 µL of 100 mM HCOOH. The technique was optimized in terms of the type of extraction solvent, voltage, extraction time, and agitation rate in a Box-Behnken design. The method was then validated according to the ANSI/ASB Standard 036, 1st edition guidelines, and all analyses were performed using an ultra-high performance tandem mass spectrometry system in the multiple reaction monitoring mode.

Results & Discussion: The optimum extraction conditions were defined as 2-undecanone as extraction solvent, 35 min of extraction time, and 40 V and 725 rpm to achieve mass transfer. Validation results showed $r^2 > 0.99$ in the 1-500 ng/mL range, accuracy and imprecision -11.8-7.1% and 5.3-19.2%, respectively, 1 ng/mL as limit of quantitation, 0.1-0.5 ng/mL as limit of detection, matrix effect -3-18%, and recovery 63-110%. Finally, the method was evaluated using the AGREEprep tool and achieved a score of 0.77, which is considered good, especially in comparison to ordinary 96-well LLE (score 0.47) or other miniaturized techniques for drug analysis, such as DLLME (score 0.5).

Conclusion: The application of EME in the prototype 96-well plate setup to a new class of NPS was demonstrated. High analyte recovery and low matrix effect were achieved with only 35 min of extraction and 3 µL of an organic solvent, thus providing a greener, simpler, and faster alternative for high-throughput NPS analysis. Moreover, SC within a wide log P range were included in this study and therefore show that the 96-well plate-EME is suited to analyze other drugs of the same class that eventually appear in the illicit drug market.

Scientific Session 4 – Alternative matrices and alcohol biomarkers

16:30 – 18:00 Tuesday, 3rd September, 2024

Chair: Yi Ju Yao, Sarah Wille

Comparison of PEth in blood and ethyl glucuronide in hair in Driving Aptitude Assessment

AB O-1

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Background & Aims: For driving aptitude assessment (DAA), the analysis of alcohol biomarkers is essential to detect alcohol consumption beside psycho-medial exploration. In Switzerland the analysis of ethyl glucuronide (EtG) in hair has been the gold standard for abstinence monitoring in DAA cases. Since October 2022, PEth 16:0/18:1 and PEth 16:0/18:2 have additionally been determined and used for further exploration of previous drinking or abstinence.

Methods: PEth 16:0/18:1 and PEth 16:0/18:2 were analysed in dried blood spots by LC-MS/MS with decision limits according to the 2022 consensus of Basel (20 and 200 ng/mL). EtG was analysed in hair by LC-MS/MS and GC-MS/MS with decision limits according to the guidelines of the Swiss society of forensic medicine (SGRM) (7 and 30 pg/mg).

Results & Discussion: In 300 DAA cases, PEth (in blood) and EtG (in proximal hair of up to 5 cm length) have been analyzed. The majority of the samples (201, 65%) were reported negative for PEth, 59 (19%) were reported as positive for alcohol consumption ("social drinking") and 51 (16%) were reported as "excessive consumption". For EtG, 134 (43%) were reported as compatible with abstinence, 70 (23%) were reported as positive for alcohol consumption ("social drinking") and 96 (31%) were reported as "excessive consumption". In 60% of all cases, PEth and EtG result interpretations were in accordance with each other. In 40% of the cases, EtG and PEth values resulted in different interpretations: in 34%, interpretation of PEth concentrations revealed lower consumption than hair analysis, only in 6% of the cases, PEth concentrations revealed higher alcohol consumption than hair analysis. Starting an abstinence phase some weeks before sampling may explain these 34% of the cases, for the other cases (6%) there might be explanations from their previous drinking history which will be discussed.

Conclusion: In many cases, analysis of PEth proves the interpretation of the EtG analysis. However, when comparing these two markers, the difference in detection windows should be considered, as PEth has a shorter detection window than EtG in 5 cm proximal hair segments. When PEth is determined repeatedly (monthly), changes in alcohol consumption habits can be detected at an earlier stage compared to hair analysis, when this is performed with 5 cm segments (representing approximately 5 to 6 months).

Automated sample preparation as leverage for high-throughput drug screening of wastewater

AB O-2

Marie Mardal, Kristian Linnet, Marie Katrine Klose Nielsen, Sys Stybe Johansen, Brian Schou Rasmussen, Ragnar Thomsen
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Background & Aims: Quantitative measurements of drug target residues (DTR) in wastewater samples can provide valuable monitoring data for population-scale drug (ab)use; an approach commonly referred to as wastewater-based epidemiology (WBE). There is a pronounced overlap between DTR monitored in routine forensic drug screening and WBE. It may thus be attainable to implement wastewater analysis in a forensic laboratory by slightly modifying existing methods. This study aimed to develop an analytical method for high-throughput wastewater analysis based on available sample preparation scripts and analytical methods. Subsequently, the developed method was validated and tested on influent wastewater samples.

Methods: As a starting point, we used calibrator, quality control, internal standard stock solutions together with LC and MS settings from a method routinely used for quantitation of common drugs of abuse in whole blood. To adapt the *in-house* methods to wastewater, sample volume and calibration range was adjusted, new quality control samples (spiked tap water at two concentration levels) were prepared, and the analyte list was modified to fit the application. Sample preparation was performed on an automated liquid handler and was based on protein precipitation. A volume of 200 µL wastewater was precipitated with 800 µL acetonitrile and then centrifuged. 700 µL of the supernatant was evaporated to dryness and subsequently reconstituted in 50 µL of 50% aqueous methanol.

The final method encompassed 15 analytes, each with paired isotopically labelled internal standards. Validation experiments consisted of three analytical runs to evaluate precision and accuracy, calibration range, and the lower

limit of quantification (LLOQ). Five analytes in the presented method were further evaluated in and passed international proficiency testing for the analysis of DTR in wastewater.

The method was applied to 24-h composite, influent wastewater from two wastewater-treatment plants from Copenhagen, Denmark: Avedøre (mostly industrial and suburban districts) and Lynetten (center of Copenhagen). Samples were collected for one week, where no special events took place. Daily loads are calculated as "mg DTR/1000 inhabitants/day", accounting for daily flow and estimated population in the catchment area.

Results & Discussion: Adjusted analytical parameters included blank matrix (whole blood to tap water), calibration range, quality control concentration levels, sample preparation injection volume (2 to 5 µL), and analyte list (ritalinic acid and citalopram added). Citalopram was included as a drug with anticipated constant intake during the week. Only DTR that *i*) consistently measured above LLOQ in wastewater samples, *ii*) performed well on the analytical method, and *iii*) were interpretable in a WBE context, were evaluated.

LLOQs in the validation experiments were 10 ng/L (benzoylecgonine, MDMA, ketamine, methadone, oxazepam, oxycodone, and citalopram), 25 ng/L (methamphetamine, codeine, tramadol, *O*-desmethyltramadol, and ritalinic acid), or 50 ng/L (cocaine, amphetamine, and morphine). The upper limit of quantitation was 5000 ng/L for all DTR. The between- and within-run accuracy of spiked QCs generally showed an underestimation, with a median accuracy of QC_{low} and QC_{high} of -10% and -6%, respectively. In the absence of blank wastewater, tap water is commonly used as blank matrix, although it is documented that this is not ideal.

The fifteen DTR were detected in all measured wastewater samples. The median {range} of measured concentrations ranged from 1690 {1095-3767} ng/L for benzoylecgonine to 36 {15-51} ng/L for oxycodone. The average ratio between tramadol and *O*-desmethyl-tramadol in wastewater samples was 1.3 {1.2-1.6}. Back-calculated DTR load during the weekend were highest in Avedøre, except for methamphetamine, MDMA, and methadone that were higher in the Lynetten samples.

Conclusion: Implementation of wastewater analysis in a forensic toxicology laboratory could be achieved with relatively minimal effort by slight modification of already available sample preparation scripts, standard solutions, and analytical methods.

The automated sample precipitation method with simple precipitation was effective for the extraction and quantitation of 15 DTR of common drugs (of abuse) in wastewater. The presented application is the first method for quantitation of common drugs of abuse in wastewater, relying on simple protein precipitation using an automated liquid handler, which allows for scalability of WBE. Possible applications include large-scale wastewater monitoring programs, aimed to collect spatiotemporal intelligence on drug consumption trends.

AB O-3

LC-MS/MS method for monitoring antiepileptic drugs in DBS. Comparison of DBS, VAMS and whole blood results.

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Background & Aims: Epilepsy is a chronic pathology affecting 50-70 million people in the world. It usually debuts in children and affects the patients for the rest of their lives. Treatment aims to avoid convulsing episodes, and while monotherapy is enough in most cases, 20-30% of the times polytherapy is required. Monitoring antiepileptic levels is essential for optimizing the efficacy and minimizing adverse effects.

The biological matrix of choice for therapeutic drug monitoring is blood, since antiepileptic blood concentrations correlate with effects. However, this requires periodic visits to the hospital and specialized personnel, and obtaining a venous blood sample is invasive. An alternative to whole blood samples are microsampling techniques (< 50 mL of blood), like Dried Blood Spots (DBS) and Volumetric Absorptive Microsampling (VAMS). Advantages of these techniques are easier and non-invasive sample collection (no need for specialized personnel), easier storage, transport and manipulation, and higher stability of the drugs.

Methods: A method was developed and validated for the determination of 8 antiepileptic drugs and 2 metabolites (carbamazepine, carbamazepine-10,11-epoxide, lacosamide, lamotrigine, levetiracetam, oxcarbazepine, 10-hydroxy-10,11-dihydrocarbamazepine, phenobarbital, phenytoin and valproic acid) in DBS samples.

Samples were cut from the card with a 6-mm punch and extracted with 200 mL of a Methanol:Water (80:20, v:v) solution in a shaker at room temperature for 30 minutes. Then, samples were centrifuged, evaporated under nitrogen, reconstituted in 50 mL of mobile phase, centrifuged again and placed into vials. For the analysis, a UPLC-MS/MS Acquity UPLC® H-Class chromatogram coupled to a Xevo®TQ-XS mass spectrometer was used. A CORTECS® UPLC® T3 (1.6 mm, 2.1x100 mm) column was chosen, with a gradient of ammonium acetate 5 mM in water and ammonium acetate 5 mM in ACN:Water (95:5, v:v). Samples were injected twice, once in negative electrospray mode (ESI-) for the analysis of valproic acid, phenytoin and phenobarbital, and another using ESI+ for all other compounds.

The method was validated following the EMA and FDA guides, including the parameters of linearity, limits of detection and quantification, carry-over, accuracy, imprecision, selectivity, matrix effect, recovery, stability, and hematocrit effect.

Results & Discussion: The total chromatographic run time was 4 minutes for ESI- compounds and 6 min for ESI+ compounds. All parameters were validated satisfactorily, with LOQs between 0.5 and 10 mg/mL and linearity from the LOQ to 10.125 – 202.5 mg/mL depending on the compound. The method was applied to 80 DBS samples from patients under antiepileptic treatment, and concentrations were compared to those in whole blood samples from the same patients, proving the usefulness of the method. The range of concentrations found in DBS was similar to the one in whole blood (WB): Levetiracetam= 3.2-33.7 mg/mL (DBS) and 3.6-29.4 mg/mL (WB); Valproic acid= 3.2-141.6 mg/mL (DBS) and 6-148 mg/mL (WB); Lamotrigine= 1.7-12.4 mg/mL (DBS) and 2.1-11.7 mg/mL (WB); Lacosamide= 4.6-11.4 mg/mL (DBS) and 6.7-8.8 mg/mL (WB); Carbamazepine= 2-9.5 mg/mL (DBS) and 2.17-10 mg/mL (WB); Phenobarbital= 13-38.2 mg/mL (DBS) and 16.6-41 mg/mL (WB); 10-hydroxy-10,11-dihydrocarbamazepine= 12.2-41.1 mg/mL (DBS) and 9.3-33.5 mg/mL (WB).

Conclusion: A method for the quantification of 10 antiepileptic compounds in DBS samples was developed, validated and applied to real samples.

Informative value of phosphatidylethanol (PEth) in post-mortem blood samples

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Background & Aims: Determination of alcohol markers in post-mortem cases can be useful to classify drinking habits and potential alcohol habituation prior to death. Phosphatidylethanol (PEth) is an alcohol marker, which can only be formed in the lipid membranes of erythrocytes in the presence of ethanol. As PEth is considered instable in whole blood, it is commonly analysed in dried blood spot (DBS) samples. Due to its high sensitivity and specificity, as well as its long detectability, PEth has already been established as a significant alcohol marker for living individuals. Its use in the field of post-mortem toxicology, however, has been scarcely investigated so far. The aim of this study was to determine the validity of PEth in routinely collected post-mortem heart blood (HB) and femoral vein blood (FB). In addition, the stability of PEth in post-mortem blood under routine storage conditions (20°C / -4°F) was assessed over a time period of 60 days.

Methods: HB and FB were collected from post-mortem specimens during medicolegal autopsies and used to create DBS on Whatman® filter paper in duplicate using 20 µL each (day 0). Six aliquots of 60 µL for each blood were pipetted into glass vials and stored in a freezer at -20°C (-4°F). Further DBS of 20 µL were created from these aliquots in duplicate on days 1, 2, 7, 14, 30 and 60. All DBS samples were stored at room temperature in a zip-lock bag containing desiccant until analysis. Sample preparation was performed by mixing the cut-out DBS with deuterated PEth 16:0/18:1 and a water/isopropanol-mixture followed by a liquid-liquid-extraction using n-hexane. The supernatant was evaporated to dryness under a nitrogen stream and reconstituted in 100 µL of the mobile phase. Analysis of PEth 16:0/18:1 and PEth 16:0/18:2 was carried out using LC-MS/MS with a limit of detection of 3.0 and 3.5 ng/mL, respectively. Initial blood alcohol was determined via GC-FID in FB for all specimens using a cut-off of 0.05 g/kg.

Results & Discussion: Blood was collected from 36 different post-mortem specimens (age: 0.5 - 87 years; male: 21; female: 15). Nine specimens showed positive initial blood alcohol (0.08 - 1.99 g/kg) and positive initial PEth values (70 – 2126 ng/mL), 20 specimens showed negative initial blood alcohol (< 0.05 g/kg) but positive initial PEth values (3.9 – 1208 ng/mL), and 7 specimens showed no initial blood alcohol and no initial PEth.

The higher the PEth values in HB, the higher the values in FB, which results in a strong correlation (correlation coefficient r = 0.97 for both homologues). However, the HB/FBratios of detected concentrations varied from 0.32 - 2.36 (mean = 1.00) for PEth 16:0/18:1 and 0.41 - 1.89 (mean = 0.98) for PEth 16:0/18:2, indicating a good comparability in total but a strong interindividual variation. Therefore, no recommendation can be made about which blood is more suitable for analysis.

In all PEth-positive samples, an increase in concentration of both PEth homologues over the period of 60 days was observed. An average increase of approximately 20% was already apparent after 24 hours. On day 60, detected concentrations of both homologues showed an average increase of approximately 70%. In 3 of the 7 specimens in which no initial PEth was detected, concentrations rose above the limit of detection during the observed time period.

Conclusion: Values of PEth in HB and FB are not comparable and show a strong interindividual variation, which is why an analysis of both blood samples seems necessary. Concentrations of PEth 16:0/18:1 and PEth 16:0/18:2 increase considerably when HB or FB are stored at -20°C (-4°F), indicating that values should be interpreted with great caution. The post-sampling formation of PEth in whole blood in the presence of ethanol has already been described in the literature, but in this study an increase and even a formation were also observed in blood samples without detectable amounts of ethanol. In conclusion, the (sole) use of PEth as an alcohol marker to evaluate drinking habits and alcohol habituation in post-mortem cases is not recommended. The use of additional markers, especially ethyl glucuronide in hair, should be considered.

ABO-5

Could ethylated phosphorylcholine be a new marker for ethanol consumption?

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Background & Aims: During *in vitro* exposure of HepaRG liver cells to ethanol, untargeted metabolomics revealed increased formation of ethylated phosphorylcholine (EtOChoP), a specific metabolite which was not reported before. To determine its potential role as a new biomarker for alcohol consumption and discover its detection window, EtOChoP was measured in whole blood, plasma, and serum and compared with established alcohol use biomarkers, such as phosphatidylethanol 16:0/18:1 (PEth), ethyl glucuronide (EtG), ethyl sulphate (EtS), and ethanol (EtOH).

Methods: Samples from 167 individuals were obtained in a medico-legal context from the University Hospital of Antwerp and analysed for PEth, EtG, EtS, and EtOH in whole blood using validated analytical methods. When available, other matrices (i.e., serum, plasma, urine and hair) were analysed for EtG, EtS and EtOH where applicable. For the analysis of EtOChoP in whole blood, serum, plasma and urine, a microplate liquid-liquid extraction was developed and validated. Briefly, to each microplate well, 100 µL sample and 250 µL of ice cold (-80 °C) methanol were added, and subsequently spiked with EtOChoP-D_5 as internal standard. After vortexing, the 96-well plate was placed on ice, sonicated for 2 min and left to equilibrate for 20 min. After centrifugation (30 min), the supernatant was filtered through a 96 filtration plate (polypropylene, 0.2 µm) and further evaporated to dryness using nitrogen (40 °C). The dried extract was reconstituted in 100 µL MeOH:H_2O (1:1) and five µL was injected in an Agilent 1290 Infinity LC/6495C triple quadrupole MS using a Restek Force Biphenyl (1.8 um, 50 x 3.0 mm) column.

Results & Discussion: All samples were analysed for EtOChoP, PEth, EtOH in whole blood, and EtG in hair. Out of 167 paired samples, 75 paired samples were analysed for whole blood, plasma and serum for EtOChoP, EtG, and EtS. Additionally, these 75 samples were also analysed for EtG and EtS in urine. Due to its novelty, three different matrices (serum, plasma and whole blood) were considered to identify the most suitable one to further analyse EtOChoP. Out of the 75 samples analysed, quantification of EtOChoP was achieved for 62 whole blood samples, and in less than half for serum. Consequently, whole blood was selected as the preferred matrix for comparing EtOChoP to the other biomarkers. This choice is further supported by the fact that PEth is measured in whole blood, and plasma and serum were not consistently available; thereby simplifying the comparison process.

In the 26 samples where the PEth concentration was below the limit of quantification (LLOQ, 5 ng/mL), 12 samples were below the LLOQ (0.05 ng/mL) for EtOChoP. In the remaining 14 samples, EtOChoP was quantified and most of the concentrations were below 0.4 ng/mL (n=12). Out of 19 samples where the concentration of PEth was >5 and ≤20 ng/mL, quantification of EtOChoP was achieved for 15 samples. In all these 45 samples (PEth ≤20 ng/mL), EtOH, EtG, and EtS concentrations in whole blood were below LLOQ (0.1 g/L, 20 ng/mL and 20 ng/mL, respectively). These results indicate that, in whole blood, EtOChOP has a longer detection window than EtOH and EtG/EtS, but future research is required to confirm this finding.

For samples showing concentrations of PEth >20 and ≤200 ng/mL (n=80, classified as social drinkers), concentrations of EtOChoP were >1 ng/mL (n=44). Moreover, for samples with PEth >200 ng/mL (n= 42, classed as heavy drinkers), most concentrations of EtOChoP were >6 ng/mL (n=34). Overall, there was a strong, statistically significant correlation between PEth and EtOChoP concentrations in whole blood, with a Spearman correlation coefficient, r_s of 0.848 ($p<0.001$).

Conclusion: The presence of EtOChoP in samples where PEth was detected, and its absence in teetotallers show that it can be considered as a promising new biomarker for alcohol consumption. The detectability of EtOChoP in different matrices is possibly different compared to established alcohol biomarkers EtOH, EtG/EtS and PEth, suggesting a

different detection window with potential usefulness to help establish drinking behaviour of an individual. Nevertheless, future studies are needed to determine the true potential of EtOChoP as an alcohol consumption biomarker.

AB O-6

Prevalence of substance use identified in oral fluid and hair within a large-scale US court-ordered mandatory drug testing population

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Background & Aims: Substance use disorders (SUDs) in the United States are wreaking havoc on the nation, as evidenced by the increase in law enforcement drug seizures and overdose deaths. A major issue contributing to overdoses is suppliers mixing substances with deadly consequences.

Approximately 65% of the U.S. prison population has an active SUD (NIDA, 2020)[1]. Court-ordered mandatory drug testing (COMDT) is frequently conducted in correctional settings to determine abstinence compliance or usage history (e.g., probation, parole, custody). Furthermore, prevalence data using oral fluid and hair testing is sparse. Currently, hair testing routinely performed at large commercial laboratories does not include testing for fentanyl and its analogues.

This observational study seeks to provide timely, evidence-based intelligence on growing rates of drug use and patterns of substance use in individuals enrolled in COMDT programs.

Methods: Phase I of this study analyzed over 400 hair specimens for fentanyl; a selection of fentanyl analogues; and other drugs such as cocaine, methamphetamine, and codeine by liquid chromatography-tandem mass spectrometry (LC-MS/MS). These hair samples were submitted from a COMDT laboratory and previously analyzed at this laboratory from November 2020 through February 2021. Any hair specimens that were positive for opioids on the LC-MS/MS were also analyzed by non-targeted high-resolution mass spectrometry. Hair specimen positivity rates in COMDT were calculated with and without inclusion of fentanyl and fentanyl-related compounds to determine the effect on overall positivity rate when fentanyl targets were included in the hair-testing protocol.

Phase II of this study was a retrospective analysis of 5 years of COMDT data from oral fluid and hair collected from 2015 to 2019 in nationally represented COMDT programs. Specimens were screened by immunoassay and confirmatory testing (LC/MS/MS, HRMS) was performed on a subset of oral fluid and all hair positive specimens. Statistical relationships such as direct statistical inference to the larger population of all COMDT specimens during same time period and during any time period; and direct estimates of the prevalence of positive drug tests among the analysis pool were determined. Specimens in the historical dataset were tested for misused substances by screening with immunoassay and confirmatory testing was performed on a subset of oral fluid and all hair positive specimens. The prevalence of positive drug tests among different demographic groups of the analysis pool and the positivity rates of oral fluid confirmation with and without fentanyl were calculated.

Results & Discussion: COMDT drug prevalence data can be compared to other prevalence data such as U.S. workforce testing. 959,237 oral fluid and 65,645 hair specimens were analyzed for misused substances representing a random, national COMDT sampling from 2015 to 2019. Amphetamines, opioids, cocaine, benzodiazepines, cannabinoids, and phencyclidine were detected. The screening positivity rate in oral fluid was 34.0% with 7.8% total confirmed, whereas the hair overall positivity rate was 56.0%. This is up to 5 times higher than oral fluid and hair in a U.S. workforce population during similar time period (10.2% and 10.9%, respectively; Quest Diagnostics, 2018) [2]. Representative drug combination positivity rates were also determined

Conclusion: This is the first large-scale drug prevalence study in a COMDT population. These results can serve as an early warning to help legal systems and public safety programs support treatment for SUDs.

[1] Center on Addiction, Behind Bars II: Substance Abuse and America's Prison Population, February 2010. https://files.eric.ed.gov/fulltext/ED509000.pdf.

[2] Quest Diagnostics Drug Testing Index, 2018 https://www.questdiagnostics.com/content/dam/corporate/restricted/documents/employer-solutions/Drug-Testing-Index-2018---web.pdf.

AB O-7

Dental calculus as alternative matrix – evaluation of drug findings in eight postmortem cases

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Background & Aims: Mineralized biofilm (calculus) develops when established biofilm on the tooth is not sufficiently removed over weeks and subsequently minerals from saliva are deposited on and in the biofilm. The result is a very resistant, stony, porous substance that can only be removed with dental tools. Archaeological findings have shown that calculus can still be collected and analyzed from skeletons that are years or even centuries old. The aim of this study was to provide insight into the mode of drug deposition in calculus and to evaluate its potential as an alternative matrix for detecting drug ingestion.

Methods: In eight toxicologically interesting postmortem cases, calculus was collected via dental scaler in addition to the usual postmortem matrices femoral vein blood, heart blood, and urine. Drugs were extracted from calculus by ultrasonication in acetonitrile from unground calculus and after pulverization (BeadBug). Both extracts were analyzed separately by liquid chromatography-tandem mass spectrometry (LC-MS/MS, Shimadzu UHPLC, Sciex QTrap 5500 mass spectrometer, Phenomenex Luna PFP column) in multiple reaction monitoring mode (MRM). Cultivated biofilm was used as blank matrix for calculus calibration.

Results & Discussion: A total of 39 drugs and metabolites were detected either in calculus, blood or urine. In one case 13 analytes were detected in calculus (12 of them in blood and 11 in urine). Suprisingly, one case showed opiates only in calculus but not in blood or urine. Moreover, piritramide, which is a rather unstable analyte, was detectable in calculus - but not in the body fluids - in two cases. This could indicate a stabilizing property of calculus as postmortem matrix. Other analytes found only in calculus were ambroxol, a metamizol-metabolite (4-AAA), levetiracetam and lidocaine (possibly due to oral intake as lozenges, drops or solutions). For most drugs, a comparably high concentration in calculus corresponds to a comparably high concentration in blood, with the interesting exception of benzodiazepines, showing high concentrations in calculus and rather low concentrations in blood. The extraction yield of unground calculus was mostly higher than that of the subsequently pulverized calculus. This indicates that drugs are deposited on the mature calculus, as well as in deeper layers, probably during calculus formation.

Conclusion: Dental calculus can be a useful addendum to the postmortem analytical portfolio especially for unstable compounds. Drugs seem to be deposited both by contact with calculus and during calculus formation, suggesting a wide time window for drug detection.

Scientific Session 5 – Hair analysis I, joint with SoHT

08:30 – 10:00 Wednesday, 4th September, 2024

Chair: Alberto Salomone, Tina Binz

SOHT O-1

Cortisol in hair as a stress biomarker in pregnant women and its correlation with demographics and clinical outcomes

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Background & Aims: Cortisol is a steroid hormone that regulates the human stress response. Exposure to stress during pregnancy can have deleterious health effects on the mother such as shortened gestation and on the risk of neurodevelopmental and cardiometabolic disease across the life course. Hair is an ideal matrix for analysis of cortisol levels because it allows for its evaluation for an extended time period, and it is drastically less affected than blood, saliva or urine by circadian variations. The goals of this study were three-fold: 1) to develop and validate an analytical method for cortisol in hair; 2) its application to determine cortisol concentrations in hair samples from pregnant women at the end of pregnancy; and 3) to correlate cortisol hair concentrations and maternal and neonatal demographics and clinical outcomes.

Methods: Due to the endogenous nature of cortisol, the analytical method was performed using 13C3-labeled cortisol as a surrogate analyte. Hair samples were decontaminated with water and acetone. After overnight drying, 10 mg of hair was snipped into small pieces (< 1 mm) and incubated in methanol in an ultrasound bath at 55°C for 90 min and at room temperature overnight. The extract was evaporated, reconstituted, filtered (PES filter vials) and analyzed in electrospray in negative mode via liquid chromatography tandem mass spectrometry (LC-MSMS). The chromatographic column was a C18 column, and the mobile phase was 0.1% formic acid in water and methanol in gradient mode. The method was applied to a cohort of 114 pregnant persons who delivered at Jack D. Weiler Hospital in the Bronx, NY between 5/11/22-1/26/23 (NICHD; 3R01HD092533-05S1 'Impact of COVID-19 and Structural Racism on Maternal Mental Health' study). Hair samples were segmented in one (n=42), two (n=3) or 3 (n=69) segments to investigate cortisol levels throughout pregnancy. Segment length varied depending on the type of sample; one-segment samples had a length of 1 to 9 cm, and the two- and three-segment samples had a length

of 3 cm. A total of 255 segments were analyzed. Chi square or Fisher's exact test were used to assess associations between categorical variables. T-test, ANOVA or Pearson's correlations were used to assess associations between categorical and continuous variables, as appropriate.

Results & Discussion: The method was linear (n=5) from the limit of quantification at 1 pg/mg to 500 pg/mg. Imprecision (n=15) was less than 18% and bias (n=15) was between -9.6 to -1.0 % at three quality control levels (3, 30 and 300 pg/mg). No matrix effect was detected (n=8), and extraction efficiency was 76% (n=8). Out of the 255 segments analyzed, most of the segments had cortisol concentrations between 1 and 100 pg/mg (n=227), between 100 and 500 pg/mg in 12 segments, above 500 pg/mg in 10 segments, and no cortisol in 6 segments. The average and median cortisol concentrations in the most of the segments (n=227) was 13±15 pg/mg and 6.2 pg/mg. Among the cases with 3 segments representing the first, second and third trimester (n=69), statistically significant differences were observed throughout pregnancy. An increase in cortisol concentrations was observed throughout pregnancy if cortisol concentrations were below 100 pg/mg, but a decrease was observed if concentrations were above 100 pg/mg. Significantly higher cortisol concentrations were observed in non-Hispanic white participants compared to other races and ethnicities, in women with higher pre-pregnancy BMI, and if they received or applied for unemployment insurance. No correlations were observed with most of the clinical outcomes, including gestational age and birthweight. However, significantly higher cortisol concentrations were measured in women who gave birth to appropriate size for gestational age newborns compared to intrauterine growth restricted neonates.

Conclusion: A sensitive and specific method for the determination of cortisol in hair was developed and validated, achieving a LOQ of 1 pg/mg using 10 mg of hair. Varying cortisol concentrations were measured in 114 pregnant women, from none detected to concentrations above 500 pg/mg. Although cortisol hair concentrations correlated with race/ethnicity, BMI, and insurance status, no statistically significant correlations were found with most of the clinical outcomes.

Advancements in forensic hair analysis using UPLC- Q-TOF and UPLC-MS/MS demonstrated via a case report concerning synthetic cathinone abuse

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Background & Aims: The primary challenge in detecting New Psychoactive Substances (NPS) lies in their rapid evolution and diversification. As manufacturers modify chemical structures to create new variants, existing detection methods may become quickly obsolete. This makes it difficult for forensic laboratories to keep their analysis protocols up to date and for drug control agencies to effectively identify and regulate these emerging substances. According to EMCCDA [1], a surge in the availability and production of synthetic cathinones in Europe is evidenced. Common examples include mephedrone and methylone. Through the generic legislation of 6 September 2017, in Belgium most NPS have been regulated thanks to a generic structure classification including the synthetic cathinones. In 2022, nearly 5000 hospital samples primarily containing various NPS were reported. Belgium is experiencing a growing prevalence of cathinone abuse. Alpha-pyrrolidinopentiophenone (α-PVP), alpha-pyrrolidino-hexiophenone (α-PHiP), but recently also alfa-pyrrolidino-cyclohexylphenone (α-PCyP) are known by their street name, Flakka. Recently Belgium recorded 121 cases of hospitalizations related to Flakka. α-PHiP or α-PHP was detected in 98% of these biological samples with occasional difficulty in distinguishing between the two substances analytically [2]. In this sense, it is imperative to establish a robust and efficient screening method for hair analysis due to its numerous advantages. Hair analysis offers a longer window of detection compared to other biological samples, allowing for retrospective monitoring of substance use over an extended period. With its ability to provide valuable insight into long-term drug exposure, hair analysis emerges as a promising tool in the identification and monitoring of NPS consumption, complementing traditional forensic methods. Additionally, hair analysis can aid in identifying trends in drug consumption, facilitating a better understanding of shifting patterns and behaviours within populations. However, given the limited sample quantity typically available in hair analysis, the routine analysis method must be efficient to maximize the utility of the sample. This study aims to enhance the detection of NPS in hair samples using Time-of-Flight (QTOF) mass spectrometry and Ultra-Performance Liquid Chromatography Tandem Mass Spectrometry (UPLC-MS/MS). An example of the workflow is given via a case report for which abuse of Flakka was suspected.

Methods: Hair samples were segmented and decontaminated with dichloromethane, water, and methanol. Twenty milligrams of hair sample were pulverized and incubated with methanol (0.05% formic acid) under sonication at 45°C for 3 hours. After centrifugation, the supernatant was filtered, evaporated, and reconstituted in an ammonium formate buffer. The same extract was analysed with the Quadrupole Time of Flight (Q-TOF) screening method and UPLC-MS/MS quantitative methods.

Results & Discussion: Although the Q-TOF routine screening method and library were initially based on a commercially available platform (Waters), collaboration with our institute's drug department has led to significant improvements and updates following trends and including NPS. This collaborative effort has greatly enhanced the reliability of routine toxicology results. In routine analysis, hair samples are systematically screened and quantified using our established methods. Despite the limited amount of hair available, utilizing the same extract for both screening and quantification enables us to maximize results, especially when detecting numerous compounds in a single extract. This approach significantly increases the likelihood of detecting a wide range of substances, including NPS. This was illustrated with recent detection of synthetic cathinones in our laboratory. The analysis of a hair sample from a post-mortem case revealed the presence of α-PVP, and α-PHiP across all segments (hair strand of 36 cm), alongside other substances. Notably, Q-TOF analysis facilitated the distinction between α-PHiP and α-PHP, providing enhanced specificity. The compounds were afterwards quantified via UPLC methods resulting in: (a) midazolam in the first segment at 8 pg/mg suggesting recent administration; (b) mirtazapine ranging from 2-6 pg/mg along the hair strand. (c)α-PVP ranging from 26 to 474 pg/mg, α-PhiP concentrations higher than the Upper Limit of Quantification (ULOQ) (1250 pg/mg); (c) increasing concentrations from root to tip were observed for pregabalin (139-1793 pg/mg), tramadol (614-higher than the ULOQ (5000 pg/mg)), ketamine (59-1103 pg/mg), and cocaine (119-925 pg/mg) and their metabolites.

Conclusion: Our study highlights the benefit of analyzing hair samples to monitor for a range of drugs as poly-drug use is a reality in case work. It also demonstrates the value to monitor trends to be able to update Q-TOF libraries and UPLC-MS/MS techniques. The ability to distinguish closely related compounds such as α-PHiP and α-PHP underscores the importance of advanced analytical methods in forensic toxicology. Due to its wider detection window of hair compared to other biological matrices, hair analysis provides valuable insights into NPS abuse patterns, assisting law enforcement and public health efforts to combat the spread of these dangerous substances.

[1] https://www.emcdda.europa.eu/publications/european-drug-report/2023/drug-situation-in-europe-up-to-2023_en.

[2] Flakka: one to keep an eye on? Severe, poly-drug intoxications involving 'Flakka': A compilation of Belgian case reports. M. Balcaen, N. De Brabanter, N. Verougstraete, C. Lyphout, AS. Decavele, O. Heylen, K. Croes, M. Degreef.

SOHT O-3

Hair analysis as an epidemiological indicator to assess the drug situation in Europe

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Background & Aims: New Psychoactive Substances (NPS) are accumulating in the illicit market and are (un)consciously used in combination with common drugs of abuse (DoA), alcohol, and pharmaceuticals thus complexing their detection in biological samples, the understanding of (in)direct health effects, and interpretation of the analyses. The EMCDDA uses several approaches like wastewater or syringe residue analysis as indirect indicators to assess the drug situation in Europe. The aim of this pioneer study was to evaluate hair testing as an effective tool for monitoring the consumption of DoA and NPS, in different at-risk populations, while combining it with an innovative harm reduction intervention.

Methods: This multicenter research took place in 2022 and early 2023 in four different countries: Italy, France, Portugal, and Lithuania. People who use drugs were approached at drug-checking services, music festivals and raves, and were asked to donate a hair sample and to fill a web-survey to report information about the substances consumed in the previous months and patterns of use.

In total, 432 samples were collected and analyzed. When the hair was longer than 6 cm, it was divided in two segments up to 12 cm. A total of 672 segments was obtained. The analyses were performed with targeted screening of more than 150 substances by UHPLC-MS/MS and untargeted screening by LC-HRMS.

Results & Discussion: In the Italian territory, cocaine (68%), cannabis (50%), MDMA and ketamine (32% each) and heroin (26%) were the most prevalent substances, while synthetic cathinones and synthetic opioids were less frequently identified. Mephedrone, buphedrone and eutylone were detected in people who declared recent use (n=3) and past use (n=9) of MDMA and amphetamines; two people tested positive for recent use of mephedrone even though they did not declare it. Ketamine, MDMA and tramadol were often identified in samples collected from individuals who did not report any exposure to these substances.

In France, a total of 229 parent drugs and their metabolites were identified. The substances most frequently found were cocaine (96%), followed closely by ketamine (94%), MDMA (93%), and cannabinoids (90%). NPS were detected in 62 individuals, encompassing 44 distinct NPS. Among these, the most commonly encountered were 3-MMC (21 cases), DCK (17), 2F-DCK (16), dimethylpentylone (10), and both 3-FPM and mephedrone appearing 8 times each.

In Lithuania, the same pattern is repeated with cocaine (27%), MDMA (26%) and cannabis (22%) being the most used while ketamine (12%) and amphetamine (8%) following. Mephedrone (n=6) was detected mainly in combination with cocaine but also alone and N-ethylpentylone in one sample together with cocaine and MDMA. 2-FDCK (n=4) was detected in three cases together with cocaine. No heroin use was detected. Lastly, trazodone (n=7) was mainly detected as a single substance apart from two cases in which it was also found 2C-B and once amphetamine. The latter one was detected only once again together with MDMA and ketamine.

An interesting aspect is noticed in Portugal: MDMA (60%) is by far the most prevalent substance followed by ketamine (47%) while cocaine and cannabis were detected at 31% each. Amphetamine (18%) and mephedrone (2%) complete the detection window. Tramadol (n=1), mescaline (n=2) and harmine (n=3) were mainly detected in combination with other drugs.

Conclusion: The identification of substances in biological matrices in diverse populations is essential to obtain an overview of the diffusion of the substances around Europe, to understand which is the drug prevalence and trade patterns followed as well as their effects on human health. While not being representative of the general population, this innovative approach based on two complementary methods provides valuable additional information about variations in use among different groups of people who use drugs. This study confirmed some of currently observed trends in drug use in Europe, with several stimulants (cocaine, MDMA) being prevalent in all participating countries, while highlighting (and confirming signals from other data sources) on the recent increase use of ketamine and synthetic cathinones.

Chiral analysis of ketamine in hair by UHPLC-MS/MS

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SOHT O-4

Background & Aims: Ketamine is usually available as a racemate and has been been used in clinic, which has important medical values such as anesthesia. However, due to its severe psychotic side effects and addictive nature, ketamine is classified as a controlled substance in most countries. Ketamine exists in two enantiomers: R-ketamine and S-ketamine. However, the enantiomers exhibit different biological activities. The S-ketamine was shown to be more potent than the R-ketamine and exhibits a higher clearance and faster anesthetic recovery compared to R-ketamine or racemic mixture. Spravato (esketamine) nasal spray was approved for treatment-resistant depression by the the U.S. Food and Drug Administration, and was also approved for marketing in China in 2023. Therefore, the prevalence of S-ketamine will increase and the demand for analytical techniques has intensified. Hair has the advantage of providing a long drug detection window, which can be useful for the retrospective verification of drug exposure in forensic toxicology and clinical toxicology. Furthermore, hair samples present desirable characteristics, such as being non-invasive, painless and relatively simple to sample, and difficult to adulterate. It can also be stored at room temperature. Therefore, the chiral analysis of ketamine in hair can differentiate the intake of illegal (racemic or a single enantiomer) drugs and clinical medications containing another single enantiomer or identify the source of the abused ketamine. The aim of this study is to develop and validate a chiral analysis of ketamine in hair using ultra-high-performance liquid chromatography-mass spectrometry (UHPLC-MS/MS) method.

Methods: To establish a UHPLC-MS/MS method capable of quantifying two enantiomers in hair, various conditions were evaluated, including the assessment of several mobile-phase compositions, elution conditions, and chiral columns. Finally, chromatography was performed at 40AT72395_TYPOTRON_Pantonefarben_Schlussblatt.pdf on a Lux Cellulose-3 chiral column (150 × 4.6 mm, 3 µm) . The mobile phase A was 20 mmol/L ammonium acetate buffer mixed with 0.1% formic acid, and the mobile phase B was methanol. Isocratic elution (20% A and 80% B) was performed at 0.3 mL/min, with a total run time of 10 min. The extraction efficiencies were compared in authentic hair samples using four extraction solvents: methanol, acetonitrile, methanol:acetonitrile (1:1, v/v) , and a 25:25:50 (v/v/v) mixture of methanol/acetonitrile/2 mM ammonium formate (8% acetonitrile, pH 5.3). The results indicated that methanol yielded the most optimal peak shapes, the most effective separation of R- and S-ketamine, and the highest extraction efficiency. The hair sample was washed three times with acetone. After drying, hair sample was cut into pieces, and 20 mg was weighed. The hair sample was then extracted with methanol utilizing cryogenic grinding. The samples were centrifuged at 9700 × g for 3 min, and 5 µL of the supernatant was injected into the UHPLC-MS/MS system. Ketamine-d4 was used as the internal standard.

Results & Discussion: The limits of detection and quantification of both enantiomers were 4 pg/mg and 5 pg/mg, respectively. The linearity ranged from 5 pg/mg to 1000 pg/mg for both analytes. Precision remained below 10%

for both analytes, while accuracy was within the range of 90.2%~109.1%. Extraction recoveries ranged from 72% to 112%, and matrix effects ranged from 51% to 104%. This method was applied to the quantifying R- and S-ketamine in 45 authentic hair samples from forensic cases. The concentrations of R-ketamine in hair ranged from 30 to 552,666 pg/mg, with a median of 751 pg/mg, while S-ketamine concentrations in hair ranged from 31 to 443,234 pg/mg, with a median of 815 pg/mg. The ratio of R-ketamine to S-ketamine was in the range of 0.8~1.4, with a median of 1.2. Analysis of 13 hair samples from individuals coming from Myanmar revealed a ratio of R-ketamine to S-ketamine ranging from 1.2 to 1.4 (n=13), with a median of 1.3, suggesting a slightly high proportion of the R-ketamine enantiomer in this region. This finding may assist in identifying the source of the abused ketamine.

Conclusion: A quantitative method for the chiral separation of ketamine has been established, which is the first time to analyze ketamine enantiomers in human hair by UHPLC-MS/MS. This method has the potential to aid in exploring the source of ketamine use (racemic or a single enantiomer). While our findings are based on a limited number of abuse cases, they nonetheless provide valuable insights into this complex issue.

SOHT O-5

Using a hair reference material as a benchmark to evaluate drug testing performance in five US hair testing laboratories and work towards harmonization and consensus.

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Background & Aims: Hair is a solid matrix and in hair drug testing, the analytes must be liberated from the hair either by extraction or digestion. Poor recovery in this process leads to lower results which cannot be compensated by an internal standard or calibrators, as they cannot be incorporated into the hair but are instead added to the surrounding liquid. It is almost impossible to accurately measure the actual recovery in hair extractions/digestions, and therefore difficult to determine true drug concentrations in hair for reference. This also makes it difficult for laboratories to determine recovery during extraction/digestion and hence to identify methods needing improvement.

National Laboratory Certification Program (NLCP) studies and the Society of Hair Testing (SoHT) PT program show wide variability in hair quantifications between hair testing laboratories. NLCP studies indicate that this variability is primarily driven by systematic biases between the laboratories. Our hypothesis is that the observed between-laboratory variability is driven by variable recovery of drug from the hair matrix in different protocols and not by inhomogeneous distribution of drug in the hair samples.

It is important that all laboratories in a regulated drug testing program get the same result for a given sample, especially when cutoff concentrations are mandated. The aim of this study was to explore the differences between laboratories by submitting the same sample, containing multiple drug challenges, to five different US hair laboratories to compare the results.

Methods: A hair reference material was provided to five US hair laboratories for analysis and results were compared between and within laboratories. The selected hair reference material was TricoCheck® CTRL H 20 multi-CONTROL from Comedical® (Trento, Italy), containing 36 drug analytes in 7 drug classes. The material was supplied as snippets which likely precluded decontamination in some laboratories.

Laboratories were instructed to quantify 14 analytes, including cocaine, benzoylecgonine, cocaethylene, norcocaine, codeine, morphine, 6-acetylmorphine, oxycodone, fentanyl, methamphetamine, amphetamine, MDMA, MDA, and THC-carboxylic acid (THC-COOH). The analysis was repeated five times in five separate analytical batches. Processing of hair included acidic and basic digestions, production of snippets and pulverization of hair. Most laboratories followed this by a clean-up step using solid phase extraction and LC-MS/MS. Liquid/liquid extractions and GC-MS were also used.

Laboratory results were compared with assigned values and acceptable ranges (±35%) provided by the manufacturer, as well as the results from the other laboratories. After analysis, laboratories were provided with a report of their results for self-assessment and potential method improvements.

Results & Discussion: Almost all the requested results were obtained. Two laboratories did not report results for all analytes as some were outside their scope. Another laboratory only reported three or four replicates for six analytes. In general, the results from three of the laboratories agreed well with those from the manufacturer with mean results for 12/14, 10/14 and 9/13 analytes within manufacturer ranges. The other laboratories appeared to have lower recovery with results for only 1/14 and 2/9 analytes within manufacturer ranges. It is encouraging that results from the three laboratories with higher recovery quantitated within the manufacturer's acceptable ranges, even though the laboratories used different methods including both digestion and extraction. That similarly high

recoveries (perhaps close to 100%) could be obtained with different extraction/digestion techniques indicate that harmonization of hair drug testing results through benchmarking is achievable.

For benzoylecgonine, cocaethylene, fentanyl and THC-COOH, laboratory results were generally higher than those of the manufacturer, indicating that the laboratories may have better recoveries than in the manufacturer testing, but could also indicate a calibration issue.

The evaluation also revealed other method artifacts that would have been difficult to discover otherwise. One laboratory reported 172% recovery for morphine and 56% recovery for 6-acetylmorphine, likely due to conversion 6-acetylmorphine to morphine during extraction/digestion. Similarly, low results for amphetamines in several labs could be caused by loss of analyte during extraction/digestion.

The laboratories were provided with a report of their results compared to manufacturer data, and a list of references with methods for optimizing recovery of different analytes from the hair matrix. The laboratories were also offered additional aliquots of the reference material to be used in method development and improvement. As a next step, we plan to follow up with more challenges to re-evaluate laboratory performance after they have had sufficient time to make improvements to their methods.

Conclusion: The study shows that similar results can be achieved even when laboratories use different extraction/digestion methods. Allowing multiple methods, as opposed to requiring a standard extraction or digestion method, enables laboratories to harmonize while retaining different scientific approaches to pursue the most cost-efficient solutions.

By comparing laboratory data to a benchmark, and reporting this back to the laboratories, laboratories could evaluate their methods and make improvements. This strategy could be further leveraged to promote harmonization between hair testing laboratories that participate in PT and accreditation programs.

Evaluation of the influence of keratin hair straightening on the detection of xenobiotics, illustrated with caffeine and endogenous GHB- preliminary study.

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Background & Aims: For several years, keratin hair straightening has gained popularity as a treatment aimed at maintaining straight, more manageable, and frizz-free hair for an extended period, typically up to 5 months, thereby enhancing its overall health appearance. The procedure involves an initial hair washing step, which facilitates the opening of hair pores, thereby promoting enhanced adherence of keratin protein to the hair shaft. Subsequently, a serum containing hydrolyzed keratin and other adjunct substances is uniformly applied until all hair strands are coated. The treated hair is then subjected to blow-drying and flat-ironing to effectively seal the keratin onto the hair structure. Due to concerns over the presence of carcinogenic formaldehyde or its derivatives in some products, which were previously utilized in the binding process of hydrolyzed keratin, these have now been substituted with non-formaldehyde alternatives, commonly referred to as safe keratin treatments (SKT).

The objective of this study was to evaluate the impact of keratin hair straightening on the detectability of xenobiotics, specifically by comparing the concentrations of caffeine and endogenous gamma-hydroxybutyrate acid (GHB) before and after the treatment. Additionally, this research aims to present the concentrations of caffeine in individual who do not consume coffee, but consume 2-3 cups of green tea daily, as well as who ingest capsules with caffeine (averaging 3 capsules daily, each containing 200 mg) and sporadically consume energy drinks containing caffeine (a few cans per month).

Methods: Two strands of undyed blond hair were collected from the occipital region of the scalp of a single individual. The first sample was obtained one day prior to a keratin hair straightening treatment, and the second sample was collected one month after the procedure. No additional hair care treatments were performed between collections, except for routine hair washing. The keratin hair straightening procedure was conducted in a professional salon using a product devoid of formaldehyde and its derivatives. Both hair strands underwent decontamination procedures, followed by division into 1.5-centimeter segments (for the post-treatment hair strand, the segment 0-1 cm was discarded due to hair growth between collections). After grinding and preparation of aliquots for each segment, the hair samples were subjected to methanol extraction for 16 hours at 60°C. To eliminate matrix effects, concentrations of caffeine and endogenous GHB were determined using the isotope dilution method employing

caffeine-d3 and GHB-d6 standards. Gas chromatography coupled with tandem mass spectrometry (GC-QqQ-MS/MS) and multiple-reaction monitoring (MRM) mode was utilized for analysis. To enhance selectivity, determination of GHB and GHB-d6 was performed after prior sample derivatization using N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide with 1% tert-Butyldimethylchlorosilane (MTBSTFA + 1% TBDMSCl).

Results & Discussion: The concentration of endogenous GHB in 1.5 cm segments of hair, before the treatment, ranged from 1.2 to 4.1 ng/mg, whereas post-treatment concentrations ranged from 0.8 to 1.3 ng/mg of hair. The difference (decrease in concentration) between pre-treatment hair segments compared to post-treatment segments ranged from 0.4 to 3.1 ng/mg.

The concentration of caffeine in 1.5 cm segments of hair, before the treatment, ranged from 1.2 to 9.2 ng/mg, while post-treatment concentrations ranged from 1.9 to 9.3 ng/mg of hair. The difference (increase in concentration) between post-treatment hair segments compared to pre-treatment segments ranged from 0.1 to 1.5 mg/g.

In all compared hair segments, the concentration of endogenous GHB decreased after the keratin hair straightening treatment, whereas the concentration of caffeine increased in each of the compared segments post-treatment. The decreasing concentration of endogenous GHB may result from the incorporation of hydrolyzed keratin into the hair, leading to increased hair mass (dilution effect). Information regarding hair care prior to toxicological analysis can significantly influence the interpretation of results, including falsely negative or underestimated results.

The increase in caffeine concentration in segments collected post-treatment likely stems from caffeine being one of the components of the product used for keratin hair straightening, and during the procedure, it may have been incorporated into the hair structure along with hydrolyzed keratin. This is valuable information in the context of using hair or scalp care products containing various substances, which, analogous to caffeine, could deposit in the hair and result in falsely elevated or falsely positive results.

Conclusion: Cosmetic procedures performed on hair, not limited to dyeing, can affect the detection of xenobiotics and alter their concentrations in hair. This appears to be particularly relevant in the context of single exposures to specific substances, such as in cases of drug-facilitated sexual assault (DFSA). Therefore, in addition to conducting toxicological tests correctly, it is crucial to gather detailed history from the individual from whom the hair sample was obtained for testing and to interpret the results appropriately based on all available information.

To confirm the obtained results, further cases should be examined, including individuals with different hair colors or individuals with dyed hair subjected to keratin hair straightening.

SOHT O-7

Analyte recoveries from intact and powdered authentic hair at different extraction conditions.

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Background & Aims: The pretreatment of hair samples is known to influence the quantitative values of drugs and metabolites because the recovery of analytes from the hair matrix differs between procedures. It is difficult to investigate absolute recovery from authentic hair samples since the true concentrations are unknown. However, exhaustive, or repeated extraction can give an idea of when the recovery has reached a plateau and comparisons between different procedures using the same authentic hair can help showing superiority of one procedure over another. So far, a standard protocol for the extraction of drugs from hair has not been proposed. In this investigation we aimed to compare different extraction conditions for a range of compounds and find a procedure working for as many compounds as possible.

Methods: A pool of authentic hair was used for the comparisons. Each experiment was performed using duplicates or triplicates of 20 mg hair that were extracted using a mixture of 500 µL formate buffer:acetonitrile:methanol (80:10:10). When powdered, four metal beads were used in each sample tube. Powdering was performed in a Lysera bead mill homogenizer (Biotage). The powdering procedure was investigated by varying agitation speed (1.0, 2.1, 3.1, 4.0, and 5.3 m/s) and extraction time (1, 3, 6, 9, 12, and 15 min) with one set of experiments also comparing against incubation at 37 °C for 18 hours with both intact and powdered hair. Before analysis with liquid chromatography time of flight mass spectrometry (LC-QTOF-MS), the samples were filtered in a 0.2 µm Captiva RC filter vial (Agilent). LC-QTOF-MS was performed with an Agilent 6546 QTOF instrument equipped with a Jet Stream interface for electrospray ionization in combination with an Agilent 1290 Infinity LC instrument. Mobile phase A consisted of 0.05 % formic acid in 10 mM ammonium formate and mobile phase B consisted of 0.05 % formic acid in acetonitrile. High-resolution separation was achieved within 12 min by stepwise linear gradient chromatography (1 % mobile phase B for 0.5 min, 5-50 % mobile phase B for 7.5 min, 50-95 % mobile phase B for

2 min, 95% mobile phase B for 1 min, 1 % mobile phase B for 1 min) at a flow rate of 0.5 mL/min on a Waters Acquity HSS T3 column (150 mm×2.1 mm, 1.8 µm) maintained at 60 °C. Data were acquired in auto MS/MS mode, m/z range 50–1,000, with a scan rate of 3 spectra per second (MS) and m/z range 40-900, with a scan rate of 2 spectra per second (MS-MS).

Results & Discussion: Using a total score >90 and a peak area >15,000 initial analyses revealed that 37 drugs and metabolites were identified in the hair pool from both intact and powdered hair incubated for 18 hours. The analytes identified were 6-acetylmorphine, acetaminophen, alimemazine, amphetamine, bensoylecgonine, caffeine, carbamazepine, citalopram, clozapine, cocaine, codeine, cotinine, desmethylcitalopram, desmethylclozapine, desmethylmirtazapine, dextromethorphan, dextropropoxyphene, didesmethyltramadol, diazepam, hydroxychloroquine, hydroxyzine, lidocaine, MDMA, methadone, methamphetamine, metoprolol, mirtazapine, moclobemide, N-desmethyltramadol, nicotine, O-desmethyltramadol, O-desmethylvenlafaxine, paroxetine, quetiapine, tramadol, venlafaxine, and zolpidem.

The mean (median) area ratio Intact/Powder was 0.73 (0.69) with a range from 0.41-1.28. A t-test showed ($p<0.05$) that 22 analytes had greater recovery for the powdered hair but for 15 there was no difference between the procedures. The following set of experiments focused on agitation speed during a 3*3 min cycle and unsurprisingly an increased speed resulted in higher recoveries for all analytes. Four analytes, paroxetine, quetiapine, venlafaxine, and O-desmethylvenlafaxine showed a maximum recovery at speed 4 m/s and then a decline whereas all other analytes reached a plateau or showed a continuous trend upwards. The last set of experiments evaluated how the extraction time affected the recovery at the chosen speed 5.3 m/s. All analytes except hydroxyzine and acetaminophen showed an increasing trend. After the maximum recovery was reached the analytes alimemazine, dextromethorphan, cocaine, mirtazapine, desmethylmirtazapine, quetiapine, and O-desmethylvenlafaxine showed a decline suggesting degradation of analytes with increasing extraction time. Of all analytes, 30 reached their maximum at 9 minutes or before whereas 6 analytes (hydroxychloroquine, methadone, metoprolol, quetiapine, and two tramadol metabolites) needed 12 minutes, and dextropropoxyphene needed 15 minutes to reach a plateau. During extraction it was noted that the temperature increased and this may have caused some analytes to degrade when the extraction time was longer than 9 minutes.

Conclusion: We conclude that a simultaneous extraction and powdering procedure at high speed during a short time may serve as a universal extraction procedure, always better or equal to exhaustive extraction from intact hair.

Scientific Session 6 – Hair analysis II, joint with SoHT

10:30 – 12:30 Wednesday, 4th September, 2024

Chair: Frank Sporkert, Marc LeBeau

Detection of pyrazolam in hair by LC-MS/MS in two drug-facilitated-crime cases

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Background & Aims: This case report describes the use of pyrazolam to incapacitate the victims and how hair analysis can aid in late reported cases. Hair analysis has been suggested as a useful matrix in drug facilitated crime cases (DFC) because of its extended detection window compared to blood or urine. Pyrazolam is a designer benzodiazepine that appeared in Sweden as a recreational drug as early as in 2012 followed by a scheduling in 2014. Nonetheless, in two suspected DFC cases during 2022, the investigation suggested that pyrazolam had been the substance used for incapacitating two victims. The event was reported to have happened in September 2022. In order to verify or refute the use of pyrazolam, hair samples were obtained from the victims in January 2023, 4 months after the event.

Methods: An initial screening of 2 x 3 cm hair segments was performed by high resolution mass spectrometry and found presumptive positive for pyrazolam. For confirmation, hair samples were segmented into 6 x 1 cm segments, S1-S6, where S1 represented the segment closest to the scalp. The segmented hair samples were decontaminated with isopropanol and three 30-minute phosphate buffer washes according to the laboratory's standard protocol. Ten (10) mg of each segment was pulverized prior to extraction. Extraction was performed by sonication for 80 minutes in extraction media (80:10:10 20 mM ammonium formate buffer:acetonitrile:methanol) followed by filtration (0.2µm Agilent Captiva RC filter). An Agilent 6490 LC-MS/MS was used for the confirmation analysis and the injection volume was 10 µL and the chromatographic column was a Waters Acquity HSS T3 (2.1 x 100 mm 1.8µm)

column held at 60°C. Mobile phase A was 0.05% formic acid in 10 mM ammonium formate and mobile phase B was 0,05% formic acid in acetonitrile. The gradient, starting at 5% mobile phase B increased to 99% in 2.9 minutes, with constant flow rate at 0.5 ml/min. Total run time was 4.4 minutes with pyrazolam eluting at 2.3 minutes. Pyrazolam was detected by two transitions (356>206/167) and internal standard was Alprazolam-D5 (314>286). The calibration range was 2 pg/mg to 50 pg/mg.

Results & Discussion: Pyrazolam was found in both victims' hair in segments compatible with an exposure 4 months prior to sampling. In one victim 5.6 pg/mg was found in segment S4, and 6.5 pg/mg in segment S5. In the other victim 4.8 pg/mg of pyrazolam was found in segment S4. Though there are no reported concentrations of pyrazolam in hair, concentrations in the low pg/mg range have been reported for other benzodiazepines in hair after single doses suggesting that the findings can be explained by a single dose. In controlled dosing studies it has also been shown that several short segments can be positive after a single dose as a result of dormant hair and that this widening of the detection window increases with time between dosing and sampling.

Conclusion: Prompted by the police investigation, we developed a sensitive method for the analysis of pyrazolam in hair. We conclude that pyrazolam could be detected in hair several months after a single exposure and that concentrations were in the low picogram per milligram range when analyzing short hair segments.

SOHT O-9

Unanticipated results of hair analysis for synthetic cannabinoids linked to worn headwear: A case report

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Background & Aims: In substance abstinence monitoring, urine and scalp hair are conventional matrices, each with distinct merits and demerits. Urine sampling is minimally invasive, while hair sampling offers an extended detection timeframe. However, hair sampling is susceptible to various forms of contamination. For instance, exposure to sidestream smoke from synthetic cannabinoid receptor agonists (SCRAs) can yield false positive results in non-users due to deposition of the compounds and specific metabolites being formed during smoking. Additionally, e.g. hand contact with drug material can lead to drug detection in hair without consumption. A case study with hair samples collected in August 2023 unveiled contamination through the wearing of a baseball cap worn during SCRA use, resulting in positive SCRAs findings in hair despite reported abstinence since July 2021. This case underscores the intricate nature of interpreting hair analysis outcomes.

Methods: A hair sample (28.3 mg, examination length: 0 – 6 cm, total length: 8.5 cm) collected during an abstinence control program underwent decontamination by washing and sequential shaking with water, acetone, and petroleum ether. The washed hair sample was treated with methanol, cut into small pieces, and subjected to ultrasonic extraction with internal standards for 3 hours. Calibration was executed using spiked blank hair matrices processed similarly. Wash solutions were combined, followed by addition of internal standards, evaporation, and reconstitution in mobile phase. A section of the client's baseball cap (approximately 6x5 cm) was rinsed with methanol. Extracts underwent GC-MS and LC-MS/MS analysis for semi-quantitative results of parent compounds and some metabolites.

Results & Discussion: The hair sample tested positive for MDMB-4en-PINACA at approximately 7.9 pg/mg, with other SCRAs - 4F-MDMB-BICA, 4F-MDMB-BINACA, 5F-MDMB-PINACA (other name: 5F-ADB), 5F-CUMYL-PEGACLONE, 5F-MDMB-PICA, ADB-4en-PINACA, ADB-BUTINACA (other name: ADB-BINACA), Cumyl-CBMICA, Cumyl-NBMINACA, EDMB-PINACA, JWH-122 and JWH-210 detected in traces. The baseball cap contained MDMB-4en-PINACA at approximately 90 ng/cm^2 and ADB-BINACA at approximately 50 ng/cm^2, with the above listed other SCRAs also confirmed. Additionally, ADB-HEXINACA, Cumyl-CBMEGACLONE and Cumyl-CH-MEGACLONE were detected at low concentrations. No SCRAs were detected in the baseball cap extract by GC-MS. Hair wash solutions supported the results, with the exception of JWH-122. Assuming that 1 cm^2 of the baseball cap surface interacts with approximately 20 mg of hair, the substance concentration in the baseball cap would at least three orders of magnitude higher than that in the hair. The findings support the hypothesis that substances transferred to the hair when the contaminated baseball cap was worn, as transfer at the parts per thousand range is plausible.

Conclusion: The spectrum of detected analytes aligns with the subject's statement, as all of these were formally reported to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) on the European drug market before the claimed abstinence period. The most recently reported SCRAs concerning this case were EDMB-PINACA and ADB-HEXINACA. They were identified in Europe for the first time in February and early July 2021.

This study suggests that such transfers may adequately explain positive hair analysis results without invoking additional exposure pathways.

Hair analysis after single and repeated short in vivo passive exposures to cannabis and "cannabis light": preliminary results

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Background & Aims: Secondhand smoke from marijuana cigarette could lead to passive exposure to Δ9-tetrahydrocannabinol (Δ9-THC), with a risk for inhaling subjects of incurring in positive drug tests. Condensation of the environmental cannabis smoke on hair represents a source of external contamination, that requires careful consideration for forensic analyses, usually performed by additional detection of metabolites or the use of cut-offs to distinguish contamination from drug use. To the best of the authors' knowledge, the impact of passive exposures to cannabis for short periods (< 30 minutes) on hair and urine analysis has not been assessed so far.

A further challenge for hair interpretation arises from products containing low-amount of Δ9-THC and rich in cannabidiol (CBD), also referred to as "light cannabis," that are being offered for sale in most of the European countries. These products could also lead to passive exposure and/or contamination of the hair matrix.

The aim of the present study was to test whether short passive exposures to the sidestream smoke of cannabis and "cannabis light" could lead to detectable levels of THC and CBD on hair and whether hair analysis could allow to distinguish between the two delivered products.

Methods: To this scope, 4 abstinent volunteers were exposed in pairs to low- (0.5%) and high-Δ9-THC (5%) passive cannabis cigarette smoking. Fifteen-minutes exposures took place inside a car with no ventilation and were repeated once a week for a month for each cannabis product. Hair samples were collected after each exposure and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) through a validated method for the determination of THC and CBD. Limit of detection and quantification were 0.007 ng/mg and 0.02 ng/mg, respectively. Samples were analyzed without and with a washing step, for a total of 64 samples. To exclude drug absorption, urines were additionally collected and tested for carboxy-THC and -CBD.

Results & Discussion: An accumulation of Δ9-THC or CBD could not be clearly demonstrated over time, considering a weekly passive exposure and routine hair washing at home. Δ9-THC was detected with concentrations ranging from 0 to 0.09 ng/mg in washed samples after exposure to high-Δ9-THC smoke. As shown by previous *in vitro* studies, Δ9-THC levels were significantly higher in samples exposed to cannabis compared to those contaminated by low-Δ9-THC smoking, when considering unwashed samples. Moreover, CBD was detected from 0 to 0.08 ng/mg in washed samples exposed to light cannabis. All urine samples tested negative for THC and CBD metabolites.

Conclusion: Our study demonstrated that a single short passive exposure to cannabis and light cannabis could result in external hair contamination leading to low but detectable levels of Δ9-THC and CBD, respectively. A distinction between contamination by cannabis and by light cannabis seems feasible *in vivo*, confirming *in vitro* studies. Although an accumulation could not be demonstrated, further studies should focus on shorter intervals of exposure, to confirm the present results.

The stability of Ethyl Glucuronide (EtG) in stored hair samples

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Background & Aims: There are many accepted advantages of toxicological hair analysis for drugs and alcohol markers, including the long windows of detection and the relative simplicity of sample collection.

Two further advantages of hair over other biological samples are the ease with which the samples can be stored – as hair samples can be stored at room temperature without additional, expensive environmental-regulating equipment – and the extended lengths of time hair samples themselves can last for. The combination of the protective cuticle layer and the keratinised structure of hair cortex means that in even in conditions favourable to decomposition, human hair can persist for decades, well past the point of other common biological matrices.

Previous studies into Ethyl Glucuronide (EtG) in urine have shown that bacterial action is a cause of EtG loss over time[1]. Studies into EtG stability in blood have shown the degrading effects of temperature and resultant putrefaction on detected EtG levels over time[2].

Whilst the stability of drug analytes in hair has been documented, the same cannot be said for EtG. Therefore, the purpose of this pilot study was to determine the effects of long term (up to 72 months) storage on the levels of EtG in hair samples.

Methods: Hair samples (n=72) which had previously been tested for EtG between 2018 and 2023 (inclusive), which had sufficient remaining hair, and for which the donors had indicated no use of strong chemical hair treatments, were selected for re-testing. All hair samples had been wrapped in aluminium foil and enclosed in paper envelopes, in dry, dark, room temperature conditions for between 6 and 72 months.

All hair samples had each been previously analysed as one 3cm section, representing an overview of approximately three months. The same sectioning process was followed for the re-tests, resulting in each sample being analysed for the same three-month period twice.

Both the original and repeat testing were undertaken using the validated, in-house LC-MS/MS EtG methodology, which is accredited to ISO/IEC 17025 standards.

The results of the analyses were statistically evaluated using the Wilcoxon Signed Rank Test (alpha = 0.05).

Results & Discussion: The results were evaluated based on the year in which the original testing took place. There was no statistical difference between the first and second test results for the samples originally tested in 2021, 2022 and 2023 (p=0.756, p=0.092, and p=0.297 respectively). There were statistically significant differences between the sets of results for the samples originally tested in 2018, 2019 and 2020 (p=0.038, p=0.026, and p=0.045 respectively). This implies that there is a point (approximately 36 months after sample collection) after which hair samples should not be tested for EtG.

Conclusion: The results indicate that, when stored in dry, dark, room temperature conditions, hair samples can be tested for EtG up to 36 months after sample collection, without a significant alteration in the level of EtG present in the hair from the time of sample collection. It is possible that one or more of the biological processes previously noted in other matrices is occurring in the stored hair samples, albeit at much slower rates.

SOHT O-12

Development and validation of an HPLC-MS/MS method for the determination of benzodiazepines in hair. Comparison of two different extractions and applications on real samples.

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Background & Aims: Benzodiazepines (BDZ) are pharmaceutical drugs that are prescribed to treat a variety of conditions. BDZs represent the most widely prescribed category of drugs in Italy and Western countries. Abuse and addiction to these substances are a real problem due to the increase in stress and maladaptive pathology and the growing availability of these substances in both the legal and illegal market. Due to their sedative effect, benzodiazepines are also used for DFC (Drug-Facilitated Crimes), such as robbery and sexual assault and usually go under the name of "date rape drugs". Many biological matrixes can be used for toxicological forensic analysis, but the hair matrix is now preferred due to its non-invasive nature, extended diagnostic window, and reliability. In addition, it allows to study of the history of drug consumption or to prove the single intake for DFSA (Drug-Facilitated Sexual Assault). This study aimed to develop and validate a method for simultaneously identifying and quantifying 38 benzodiazepines and metabolites in HPLC-MS/MS. Furthermore, two different hair sample extraction procedures were compared

Methods: Hair samples were cut into small segments and washed twice with methanol and diethyl ether. 25 mg of hair were used for each extraction procedure and the HPLC-MS/MS conditions were the same for both procedures. The extracts were centrifugated and part of the supernatant (3 µL) was injected into an LC-MS/MS (Shimadzu Nexera-AB Sciex Citrine). Gradient elution was performed on Synergi Hydro-RP 100 A° Phenomenex in 8 minutes with the mobile phases composed of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The analytes were ionised using positive electrospray mass spectrometry and then detected by multiple reaction monitoring (MRM). In one extraction procedure, incubation with 500 µL of M3 ® (Comedical) and internal standards was performed on chopped hair at 100 °C for 60 min. In the second extraction procedure, incubation with methanol and internal standards was performed on pulverised hair overnight at 50°C. The supernatant was evaporated and reconstituted in 500 µL of M3 ®. Validation was performed in three different analytical sessions with three replicated per day using spiked hair samples in a range of concentrations from 2 pg/mg to 200 pg/mg (8 calibrators) and quality control

Results & Discussion: The M3 extraction procedure obtained computed LODs values between 0.8 and 9.3 pg/mg, while the methanol extraction method showed higher theoretical LODs (starting from 3.7 pg/mg) when excluding

the worst analytes. The majority of experimental LOQs are the same for both procedures. Precision intra- and inter-day was calculated for both methods. Intra-day precision ranged from ± 20% (CV%) for most of the analytes while for inter-day some are more than ± 20%, especially at lower concentration. Accuracy intra- and inter-day for most of the analytes meets the criteria of acceptability (± 20%). The matrix effect for M3 extraction, for most analytes is within the acceptable criteria (%ME from 85% to 115%) while, for methanol extraction, %ME values were acceptable only for 10 analytes. Accuracy was tested both by internal H20 Comedical control and by external quality control with VEQs from ARVECON. The z-score results of the VEQ were acceptable. Given the satisfactory accuracy results, it was decided to verify the extraction protocols' ability to extract the real samples by performing a second extraction on the residue of the previously analysed real samples. The second extraction did not reveal the significant presence of the analytes searched.

Conclusion: 50 real samples of people for whom benzodiazepine analysis was requested and were screening positive, were used to create 10 hair pools. Each pool was divided into six aliquots (for a total of 60 aliquots) and then divided into three aliquots for each extraction procedure, to have statistically significant values to compare the two extraction efficiencies. The method has demonstrated, on real samples, the ability to detect low concentrations of benzodiazepines below 10 pg/mg. The extraction protocols (M3 and methanol) have been effectively validated and proven to be interchangeable for concentrations above 10 pg/mg. The M3 protocol is also suitable for positivity at concentrations between 2 and 10 pg/mg. The analytes found most frequently were 7-Aminoclonazepam (8 M3 (2-800 pg/mg); 5 Methanol (16-760 pg/mg)), Bromazepam (4 M3; 3 Methanol), Clonazepam (7 M3 (3 – 150 pg/mg); 5 Methanol (3-124pg/mg)), Delorazepam (7 M3 (5- 117 pg/mg); 4 Methanol (20- 120 pg/mg)), Nordiazepam (6 for both (18-361pg/mg M3 - 37-373 pg/mg MeOH)) and Temazepam (4 for both (4-18 pg/mg M3; 3-23 pg/mg methanol))

Endocannabinoid and steroid levels during and after pregnancy in fingernails of mothers and their newborns

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Background & Aims: Excessive stress exposure with associated high levels of cortisol as well as stress-protective biological factors, such as endocannabinoids (eCBs), play an important role in human pregnancy and birth outcomes. However, it is unknown to what extent the combined assessments of these biomarkers are useful to predict preterm delivery or may even help to develop new interventions. An elegant and highly innovative way to measure chronic stress exposure is through steroid and eCB analysis within the keratinized matrix nail. Here, a pilot study was conducted to investigate the levels of these biomarkers during and after pregnancy in mothers and their newborns.

Methods: A total of five mothers and three newborns were included in this study. Maternal nail samples were collected during pregnancy (gestational weeks 22, 24, 26, 28, 32, 37, 42) and after birth (days, weeks and month postpartum). For newborns, the first six nail cuts after birth were collected, pooled and analyzed together. Briefly, nails were washed, clipped into smaller parts, milled and extracted for an hour with methanol in an ultrasonic bath. Afterwards, a fully automated supported liquid extraction on a Biotage® Extrahera (Uppsala, Sweden) workstation was performed. A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was used for the simultaneous identification and quantification of five steroid hormones (cortisone, cortisol, androstenedione, testosterone, progesterone) and four eCBs (anandamide (AEA), 2-arachidonylglycerol (2-AG), oleoylethanolamide (OEA), palmitoylethanolamide (PEA)). The method had been fully validated according to international guidelines.

Results & Discussion: All five steroid hormones and all four eCBs were detected in the nails of mothers and newborns. The median cortisol concentration in the nails of mothers was 4.3 pg/mg, for cortisone it was 12.3 pg/mg, for testosterone 0.5 pg/mg, for progesterone 16.3 pg/mg, and for androstenedione 1.4 pg/mg. Elevated concentrations of progesterone were found during pregnancy, which decreased postpartum. The corticosteroids cortisol and cortisone were relatively stable during pregnancy but showed generally lower levels after birth. The median for 2-AG was 15.4 pg/mg, AEA 0.7, OEA 2382 pg/mg and PEA 668 pg/mg. For the eCBs, an increase in concentrations was measured in gestational weeks 37 and 42. A return to lower levels was observed postpartum. For newborns, the first six nail cuts showed a median of 3.4 pg/mg for cortisol, 8.9 pg/mg for cortisone, 2.2 pg/mg for testosterone, 0.5 pg/mg for progesterone, 25.9 pg/mg for 2-AG, 1.7 pg/mg for AEA, 1388 pg/mg for OEA and 356 pg/mg for PEA.

In this pilot study, it was shown that the established method is suitable for the retrospective monitoring of cumulative eCB and steroid hormone levels in nails. ECB signalling is known to play an important role in embryo development and labour. An increase of AEA and 2-AG in maternal nails was observed in the third trimester. This elevation might play a role in spontaneous onset of labour, as eCBs have labour-promoting effects. Progesterone is well known to increase during pregnancy which was also visible in consecutive fingernail analyses. The interaction between the eCB system and corticosteroids needs further investigation since it is hypothesized that eCB signalling may inhibit glucocorticoid activation.

Conclusion: Nail samples can serve as a suitable matrix for the retrospective monitoring of cumulative eCB and steroid hormone levels during pregnancy and postpartum. The combined assessment of eCBs and steroids from nails can provide valuable insights into the interplay between chronic stress exposure and stress-protective mechanisms in mothers and their infants during pregnancy and postpartum.

SOHT O-14

Hair analysis of critically ill pediatric patients undergoing opioid treatment with focus on fentanyl derivatives and metabolites

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Background & Aims: The interpretation of hair analysis results has always been a challenge both forensically and clinically. This is especially true for results of hair with pediatric origin, due to differences in the hair anatomy compared to adult hair. In this context, reliable reference data regarding the incorporation of specific substances into hair poses an invaluable resource for experts. For fentanyl and its analogues (fentalogs) such data in literature is currently mainly based on forensic cases, in which multiple unknown factors such as type and quantities of the administered substances, the route of exposure, as well as patient specific parameters play a role. The objective of this research study is to investigate hair samples of a cohort including 150 children from 0 to 13 years of age that underwent opioid treatment with fentanyl, alfentanil, sufentanil, remifentanil or other more traditional opioids in a clinical setting. With the data obtained, we aimed to gain insights into the concentrations and distribution of opioids in children's hair. For this purpose, a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed and validated, targeting the mentioned opioids and selected metabolites.

Methods: This research study has a monocentric, non-interventional, prospective design. Hair samples from patients (newborns, children and adolescents) admitted to the Pediatric Intensive Care Unit (PICU) at the University Children's Hospital Zurich and exposed to medically approved opioids were collected. The samples were obtained from the back of the head in a standardized manner and processed using a two-step extraction procedure, followed by a targeted LC-MS/MS (QTRAP® 7500+) analysis. The analysis was performed in multiple reaction monitoring mode screening for fentalogs (fentanyl, remifentanil, sufentanil or alfentanil) and certain metabolites (4-ANPP, norfentanyl, betahydroxyfentanyl, norsufentanil and remifentanil-acid), as well as other more traditional opioids. The used method underwent full validation in terms of selectivity, linearity, limit of detection (LOD), lower limit of quantification (LLOQ), accuracy, precision (intra- and inter-day precision and accuracy), specificity (including matrix effect) and recovery (extraction efficiency).

Results & Discussion: The developed LC-MS/MS method showed good selectivity and sensitivity for all analytes. For fentalogs and metabolites, lower limits of quantification (LLOQ) were 0.1 pg/mg for each, and 1 pg/mg for fentanyl. Extraction efficiencies were above 80% at all three measured quality control concentration levels. Values were acceptable in terms of the bias (within ±20%) and imprecision (<20%) and ion suppression/enhancement among the different sources did not exceed ±25%. To date, a total of 104 pediatric hair samples has been collected and analyzed. Due to the small sample quantities (median: 4.30 mg), the hair samples were processed without prior decontamination or segmentation. All opioids administered to the patients during their stay in the PICU, as well as the targeted metabolites (except remifentanil-acid) could be detected in the hair samples. To the best of our knowledge, this was the first time the present fentalogs and metabolites were qualitatively and quantitatively determined in children's hair. Out of 90 cases involving fentanyl treatment, fentanyl was quantified in 60 cases (75%), in concentrations ranging from 1.03 to 4701 pg/mg and a dose concentration correlation (Spearman $r = 0.53$, $p < 0.0001$) could be observed. In some cases, the metabolites of fentanyl 4-ANPP (9 cases), norfentanyl (30 cases) and β-hydroxyfentanyl (44 cases) were quantified. Sufentanil was quantified in 46 out of 81 (56.7%) cases involving treatment, in concentrations between 0.12 and 8.64 pg/mg. Its metabolite norsufentanil was quantified in 21 cases with concentrations ranging from 0.10 to 2.43 pg/mg. Norsufentanil was previously detected in other human sample

matrices such as urine but never before in hair. Remifentanil (detected in 4 out of 11 treated cases) and alfentanil (detected in 2 out of 2 treated cases) were measured in concentrations between 0.10 and 0.43 pg/mg and 0.19 and 0.22 pg/mg, respectively. The metabolite remifentanil-acid could not be detected. This may be due to concentrations below the LLOQ or to the fact that acidic substances are generally less effectively incorporated into hair.

Conclusion: A sensitive and specific method was developed and validated for simultaneous quantification of clinically used synthetic and classical opioids in hair by LC-MS/MS. For the first time fentanyl, sufentanil, remifentanil, alfentanil and their metabolites were quantified in children's hair. The findings of this study will lay the basis for further applied research questions and will presumably serve as reference for various applications in hair testing. In a forensic context, the findings could help in the interpretation of NSO positive hair results in specific cases or larger prevalence studies in adult and especially pediatric populations. In a clinical context the study results could be used for further applied research studies in the context of therapeutic drug monitoring.

SOHT O-15

Retrospective evaluation of novel synthetic opioids and xylazine chronic intake by post-mortem hair analysis

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Background & Aims: In recent years, an increase number of fentanyls-related deaths have been reported in recreational use/abuse and therapeutic practice, particularly in the United States [1]. Indeed, fentanyl and its derivatives (non-pharmaceutical fentanyl, NPFs) represent the largest group among synthetic opioids. In addition, since the mid-2010s, an increasing number of unintentional overdose deaths involving opiates and/or fentanyl showed the presence as adulterant of xylazine, a veterinary drug able to worsen hypotension, central nervous system, and respiratory depression caused by opiates [2].

On the basis of above evidence, the need to monitor the diffusion of fentanyl, NPFs and adulterants among the population is a fundamental pursuit in forensic toxicology. In this context, hair analysis can provide important information regarding previous intake/exposure to xenobiotics. The present study focused on the application of a novel UPLC-MS/MS method for the detection and quantification of fentanyl, NPFs, and xylazine, in hair samples from post-mortem cases with the aim of retrospectively evaluate the use of these drugs among the studied population.

Methods: Hair samples (n = 250) were obtained from post-mortem cases with different causes and manners of deaths collected under the jurisdiction of the Jefferson County Coroner/Medical Examiner Office from January 2023 to March 2023.

Hair from scalp (n=191) and, when they were not available, from pubis (n=59), were decontaminated and about 50 mg were cut into small segments and spiked with of a mixture of fentanyl-D5 and xylazine-D6 to a final concentration of 200 pg/mg. Sample were then treated with 1 ml of 0.1M HCl (overnight at 45°C).

After neutralization and centrifugation, the supernatant was SPE extracted. Separations were performed by using a model I-Class ACQUITY UPLC system (Waters, USA) provided with a Force Biphenyl (2.1 × 50 mm, 1.8 µm, Restek Corporation, USA), kept at 45°C. Mobile phase A was composed of water and formic acid 0.1%, phase B consisted of acetonitrile. The samples were eluted with a linear gradient from 5 to 70% of solvent B, lasting 5 min. The liquid chromatograph was coupled with an API 6500 QTrap mass spectrometer (AB Sciex, Framingham, MA, USA) monitoring the MRM transitions for the following analytes: fentanyl, norfentanyl, ß-hydroxy fentanyl, acetyl fentanyl, acetyl norfentanyl, despropionyl para-fluorofentanyl, 4-aminophenyl-1-phenethylpiperidine (4-ANPP), carfentanil, norcarfentanil, ocfentanil, furanyl fentanyl, U-47700, xylazine, flunitazene, etodesnitazene, metonitazene, protonitazene, N-piperidinyl etodesnitazene, N-pyrrolidino etodesnitazene.

Results & Discussion: Among the studied population, n=129 of the analyzed hair samples (52%) tested positive for fentanyl either alone or with its main metabolites (norfentanyl, beta-hydroxyfentanyl and 4-ANPP).

Fentanyl hair concentrations ranged from 4 pg/mg to a concentration higher than 10 ng/mg (mean value 1697 pg/mg, median 194 pg/mg).

Regarding fentanyl metabolites, norfentanyl was detected in n=69 cases (53% of the samples positive for fentanyl) of which fentanyl was not present in only 3 cases. Norfentanyl concentrations ranged from 0.9 pg/mg to concentrations higher than 10 ng/mg (mean value 428 pg/mg, median 24 pg/mg). Beta-hydroxyfentanyl was present in n=35 hair, with concentrations from 3.3 to 2,350 pg/mg (mean value 380 pg/mg, median 116 pg/mg). 4-ANPP was detected in n=70 cases, corresponding to about 54% of cases in which fentanyl was measured. 4-ANPP concentrations were in the range 9-8,401 pg/mg (mean value 516 pg/mg, median 96 pg/mg). Both beta-hydroxyfentanyl and

4-ANPP were detected only in hair containing also fentanyl. In n=23 cases, in addition to fentanyl and its metabolites, acetylfentanyl and/or despropionyl parafluoro-fentanyl were also detected. The concentrations of acetylfentanyl and despropionyl parafluoro-fentanyl ranged from 3 to 431 pg/mg (mean value 104 pg/mg, median 22 pg/mg) and from 5 to 1385 pg/mg (mean value 129 pg/mg, median 25 pg/mg), respectively.

It is worth mentioning that 1 of the 250 cases tested positive for protonitazene, a synthetic opioid recently spread across the illicit market. Moreover, xylazine was detected in n=48 hair samples (19.2%). The prevalence of synthetic opioid positive cases was significantly higher than anticipated considering that the cause of death in many of these cases was not related to synthetic opioid (54 out of 130 cases; 41.5%).

Conclusion: In the studied population, fentanyl was the most frequently detected synthetic opioid. Moreover, acetylfentanyl and despropionyl parafluoro-fentanyl were also present in many samples. These data are consistent with the epidemiological data on the spread of NPFs in the geographical area of hair collection. In addition, the present study highlighted for the first time the detection of xylazine in hair, thus providing important information also about the xylazine emerging threat.

The results of the present study confirm the usefulness of systematic hair testing in postmortem investigation, providing helpful information with potential substantial repercussions on public health.

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[2] [2] Friedman J, Montero F, Bourgois P, et al (2022) Drug Alcohol Depend (2022): 109380.

SOHT O-16

Hydroxyketamine and hydroxynorketamine isomers can be detected in hair of ketamine users

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Background & Aims: Ketamine (K) and its initial N-demethylated metabolite, norketamine (NK), are extensively hydroxylated to a number of steroisomeric hydroxyketamine (HK) metabolites and positional and stereoisomeric hydroxynorketamine (HNK) metabolites. NK is also converted to dehydronorketamine (DHNK). HNK compounds have recently attracted scientific interest because, although they have no anesthetic effects, they do have antidepressant properties.

K hydroxylated metabolites are usually present and detectable, together with other K metabolites, in blood/plasma/serum, and urine samples. However, they are usually not detected in hair samples, due to the known lower incorporation of polar metabolites into the hair matrix than the parent compounds.

Here we describe the detection of HK and HNK metabolite isomers in different hair samples from moderate and heavy K users, by liquid chromatography - high-resolution accurate-mass Orbitrap mass spectrometry (LC-HRAM-Orbitrap-MS), after comparison with results from urinalysis of some other ketamine users.

Methods: Urine samples (300 mcL each) from subjects who had previously tested positive for ketamine were subjected to protein precipitation with 300 mcL of cold acetonitrile:methanol 2:1 (v/v) in the presence of the internal standard (IS) Ketamine-D_4.

Hair samples (20 mg) from five K users, other than the previous ones, were decontamined and pulverized with a ball mill, and, after the addition of the ISHair samples (20 mg) from five K users, other than the previous ones, were decontamined and pulverized with a ball mill, and, after the addition of the IS Ketamine-D_4, extracted with 3 mL of a methanol/trifluoroacetic acid (9:1, v/v) mixture, sonicating for 1 h and heating at 70 °C for 2 h.

Urine and hair sample extracts were subjected to LC-HRAM-Orbitrap-MS analysis using a Thermo Scientific Ultimate 3000 UHPLC system equipped with an Accucore Phenyl Hexyl reversed-phase analytical column coupled to a Thermo Scientific "Q-Exactive Focus" Orbitrap mass spectrometer instrument. MS acquisition was performed in full scan (*m/z* 70-1000) positive-ion mode at a resolution of 70,000, and subsequent data-dependent acquisition (dd-MS^2) confirmation mode (resolution 17,500, isolation window 3.0 *m/z*, isolation offset 1.0 *m/z*, normal collision energies 17.5, 35.0, 52.5 eV), according to an inclusion list of five exact mass values, calculated from the elemental composition of MH^+ ionic species, at *m/z* 238.0993 (K), 224.0837 (NK), 222.0680 (DHNK), 254.0942 (HK), 240.0786 (HNK).

Results & Discussion: Besides K, NK and DHNK, a number of HK and HNK isomers were detected in all urine samples analysed, with relative abundances varying according to individual characteristics (drug use, metabolism, sample dilution, sampling times, etc.). Likewise, in addition to K, NK, and DHNK, some hydroxylated metabolites were iden-

tified in hair samples according to the increasing K hair concentration (0.54, 1.1, 5.5, 16, and 35 ng/mg in samples 1, 2, 3, 4, and 5, respectively), and the likely extent of K exposure. In particular, HK and HNK compounds were not detected in hair samples 1 and 2. Some of the hydroxylated metabolites began to be detectable in sample 3. A number of HK and HNK isomers were detected in samples 4 and 5 from the same heavy K user.

Identification of the hydroxylated metabolites was based on evaluation of their chromatographic behaviour compared to the parent compound, accurate mass measurements of their MH^+ ions in full scan conditions, evaluation of their characteristic MH^+ isotopic patterns (considering that they are all Cl-containing compounds), accurate mass measurements of MH^+ collision-induced product ions. Tentative discrimination of alcoholic and phenolic structural isomers of HK and HNK metabolites was carried out.

Conclusion: For the first time, to the best of our knowledge, HK and HNK isomers have been identified in hair samples from ketamine users. These findings demonstrate that HK and HNK metabolites can be incorporated into hair following ketamine consumption. According to the preliminary results of this study, the incorporation processes of hydroxylated metabolites may be directly related to the extent of K exposure. HK and HNK metabolites can, at least in some cases, be assessed as markers of active K consumption.

Scientific Session 7 – Drugs in driving and on-site testing

08:30 - 10:00 Thursday, 5th September, 2024

Chair: Nikolas Lemos, Marilyn Huestis

DUI O-1

Validation of an LC-MS/MS method for screening 340 classical Drugs of Abuse (cDoA), NPS, and pharmaceuticals: Application to the analysis of residues from used syringes within the ESCAPE network

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Background & Aims: Drug injection increases overdose and infectious disease risks. Self-reported surveys on these substances often face reporting delays and lack analytical confirmation. ESCAPE (European Syringe Collection and Analysis Project Enterprise), a sentinel network, analyses used syringes in 17 EU cities to provide localized data for harm reduction (HR) interventions. To identify primary substances injected in Paris in 2023, we developed and validated an LC-MS/MS method combined to a robotic sample preparation for screening 340 cDoA, NPS, and pharmaceuticals

Methods: One hundred forty-nine syringes were collected from automatic syringe dispensers across 5 Parisian sites: Gare du Nord (GDN), Gare de Lyon (GDL), Gare St Lazare (GSL), Gare Montparnasse (GMP), and RER Kennedy station (SRK). The syringes were rinsed with 1 mL of MeOH. An automated sample preparation was completed in 45 minutes using a TECAN EVO 100 robot. A 10th dilution of the samples in MeOH was performed on a 96-well deep well plate. On a second plate, the robot dispensed 20 µL of a deuterated internal standard solution, 20 µL of the previously diluted sample, and 160 µL of 2mM formate buffer. The plate was then analyzed by LC-MS/MS on a TSQ Altis (Thermo Fisher®) in MRM mode. A single-point calibration (100 ng/mL) was used and Trace Finder software was configured for automatic result discrimination using the LOD as the reporting threshold

Results & Discussion: The reporting threshold was evaluated at 1 ng/mL for all compounds, allowing for the detection of trace substances (corresponding to 0.01% purity). 92% of syringes (n=137) tested positive for one of the 42 detected substances. Cathinones emerged as the most prevalent group, accounting for 60% (n=88) of the substances identified, with chloromethcathinone (CMC, n=81), methylmethcathinone (MMC, n=42), and chlorodimethylcathinone (CDMC, n=20) being the most common. Opioids, making up 43% (n=64) of the findings, and cocaine, constituting 25% (n=38), followed in prevalence. Cathinones and cocaine injection appears to be common during chemsex in France, as indicated by the 2023 national survey conducted by SFTA. Opioid use is mainly associated with heroin injection.

The results highlight distinct consumption patterns across Paris and the need for tailored HR interventions. For example, CMC is prevalent at GSL, GDL, and SRK, while cocaine is more commonly used at GDN and GMP. Additionally,

94 syringes (63%) contained 2 to 16 substances, indicating potential syringe reuse and increased risk of infectious diseases. However, some trace-level substances could result from blood contamination during injection.

The 2020-22 ESCAPE campaigns revealed regional drug use trends across Europe, with our results confirming Paris's preference for cathinones (along with Budapest), opioids, and cocaine. The injection of cocaine and cathinones has been linked to higher HIV and HCV transmission due to frequent use and shared paraphernalia

Conclusion: The developed screening method by LC-MS/MS, combined with an automated sample preparation and result generation processes, enabled rapid analysis of large sample sets, identifying the most frequently injected substances in Paris. Understanding the substances injected, along with related risks like multiple substance use or material reuse is crucial to optimize HR interventions and law enforcement strategies

DUI O-2

Preliminary pharmacokinetic and psychophysical data after controlled inhalative and oral consumption of hexahydrocannabinol (HHC)

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Background & Aims: The semisynthetic cannabinoid hexahydrocannabinol (HHC) has gained great popularity as alternative to tetrahydrocannabinol (THC) since mid of 2022. In most countries, it has now been banned by national regulations. Research data on pharmacokinetics and metabolism are still very limited. Previous studies have highlighted the presence of HHC in biological samples from drivers suspected of drug consumption, underscoring its potential impact on road safety. Aim of this study was the collection of data regarding pharmacokinetics and psychophysical effects after HHC intake. Additionally, immunological tests for different sample specimens were evaluated for the detection of HHC consumption.

Methods: So far two participants (I1: female, 80 kg, non-smoker; I2: male, 90 kg, regular smoker) consumed three puffs from an HHC vape, and one volunteer (O1: female, 88 kg) ate a fruit gum with 25 mg of HHC. Different psychophysical tests were performed. Additionally, pupil size, rotary nystagmus, and subjective "high-feeling" were monitored. Immunological tests (DrugWipe® 5S for saliva, SureStep™ for urine, and Immunalysis™ ELISA for serum) were conducted at various time intervals post-consumption. Serum (up to 24/48 hours), urine (up to five days), saliva (up to 48 hours), and hair (after one month) samples were collected at different relevant time points and analyzed by LC-MS/MS.

Results & Discussion: Maximum HHC serum concentrations (=c_{max}) for inhalative HHC intake were 18.0 ng/mL (I1) and 172.8 ng/mL (I2) after 3 and 6 minutes (=t_{max}), respectively. Two hours after oral HHC intake, a maximum serum concentration of 16.4 ng/mL was quantified. Variations in smoking techniques resulted in different c_{max}. The t_{max} values corresponded to known data for THC.

Results in the psychophysical tests varied between individuals. Two individuals displayed no (I1) or only minor (O1) impairments after HHC consumption. However, one participant (I2) exhibited significant impairment during the acute phase after inhalative HHC intake, requiring the cancellation of initial tests. A maximum subjective "high-feeling" of 0, 8, and 7 (scale 0-10) was reported by participant I1, I2, and O1, respectively.

The DrugWipe® 5S was positive in all three cases directly after consumption for a short time interval, presumably due to residues in the mouth. However, only in one case with oral HHC intake, it was positive again for a short time three hours post-consumption. Therefore, HHC mono-consumption does not seem to be detected sufficiently with this test. The SureStep™ urine test was positive in all three cases. After inhalative intake, the urine test remained positive for a maximum of six hours. Oral consumption led to a positive urine test result up to 72 hours. Therefore, this test can detect HHC mono-consumption, but may lack in detection of the acute phase. The Immunalysis™ ELISA for serum was able to detect the acute phase in all cases and remained positive for a maximum of 48 hours. However, in this study only a single and relatively low dose was tested. For higher doses or multiple use of HHC products detection times with immunological tests may be longer and impairment may be more significant.

Conclusion: These data highlight the diverse pharmacokinetic profiles of HHC depending on the route of administration and individual factors. The results of the psychophysical tests varied a lot between individuals. However, even if no or only minor impairment was noticeable in two cases, HHC could still be detected in serum of all participants. Immunological saliva tests demonstrated limited sensitivity in detecting mono-consumption of HHC, while urine tests exhibited challenges in detecting the acute phase. Further, quantitative analysis of all collected matrices as well as a wide metabolite screening with distinct focus on the diastereomers of the individual analytes are ongoing.

The development of a D-9-THC colorimetric breathalyzer using 3D printing manufacture

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Background & Aims: According to the 2021 World Drug Report cannabis continues to be one of the most widely used drugs worldwide. UNODC estimates that almost 4% of the global population aged 15–64 years used cannabis at least once in 2019, the equivalent of some 200 million people. Currently, in the United States, 31 states and the District of Columbia have legalized non-medical cannabis. In 2020, the legal marijuana market in the United States recorded total sales of $18.3 billion. The high profit in part can be attributed to the 2018 Farm Bill, which defines hemp as a cannabis plant or finished product containing 0.3% or less decarboxylated Δ9-THC and removed hemp from the controlled substances list. As more and more products containing Δ9-THC are becoming available, concerns about the public health consequences of cannabis use have increased.

As a result of Δ9-THC ability to impair performance and decrease the ability of drivers, and the current lack of correlation between driving impairment and Δ9-THC concentrations in blood, and the difficulty of creating rapid results in situ increases the necessity of a quick and portable detection method to identify Δ9-THC in non-invasive samples from drivers is imperative. Due to the popularity of alcohol breathalyzers, exhaled air was chosen as the matrix for the Δ9-THC detection. The concentration of Δ9-THC in the breath is estimated to be as high as 250 parts per trillion (ppt); a challenging detection limit that a THC breathalyzer developer must consider. However, the Δ9-THC concentration in the breath that monitoring platforms can detect might be higher since some of the generated Δ9-THC during smoking will be carried by aerosolized particles and deposited on the lung tissues. These particles can be removed by exhalation and be detected in breath. The goal of this project was to develop a cartridge-based colorimetric reaction for the creation of a breathalyzer for the detection of Δ9-THC in breath samples.

Methods: The development of the D9-THC breathalyzer was based on the application of an additive manufacturing solid device made by 3D printing. Five different 3D polymerizable resins and three different cannabinoid-specific dyes were analyzed. Resins were treated with different concentrations of Fast Blue B, Fast Blue BB, and Fast Blue RR dyes, followed by treatment with 10 µg of Δ9-THC for the optimization of best resin/dye/dye concentration (1, 2, 4, and 6% w/w) set. Cartridges were printed using an Anycubic 3D printer®. Color formation was captured using a ballistic microscope coupled to a high-resolution camera and the intensity of the color was analyzed using the software ImageJ®. The solubility of the dye in the resin, the analytical signal obtained, the stability of the dye, and the influence of the shape of the cartridge were evaluated.

Results & Discussion: Five resins commercially available were tested, including the AnyCubic 3D resin suggested by the printer vendors. The white Creality 3D resin showed the best signal for the same concentration of D9-THC among all resins. The solubility of the dyes also showed to be better in the Creality resin when compared to the other four resins tested. Among the dyes, Fast Blue B was shown to be the best option as it results in higher signals than the signals given by Fast Blue BB and Fast Blue RR for the same amount of D9-THC. The concentration of dyes was relevant for the analysis as higher concentrations showed saturation of the dye in the resin resulting in smaller solubility. The concentration of 1% was chosen not because of the best signal given but because of the good solubility of the dye in the resin at this concentration, without significant loss of signal intensity. The cartridge shape was shown to be relevant to the stability of the reaction. However, a circular shape is the target shape due to the air collection system that will be attached to the cartridge to form the breathalyzer prototype. A current validation of the parameters is being performed and preliminary results showed the capacity of the cartridge to react positively to a concentration of 0.01 µg of D9-THC. Due to the nature of the reaction, no cross-reactivity with CBD is expected, and indeed, this is one of the advantages of this prototype. Achieving lower concentrations is extremely important as the concentration of D9-THC in exhaled air is described to be 1 ng/30 L of exhaled air.

Conclusion: In recent years, due to the increasing demand for both product complexity and multi-functionality, many new materials have been tested for 3D printability and the forensic science application is among them. The results obtained in this work are the initial fundamental chemical foundation needed for the construction of a reliable semi-quantitative breathalyzer device to be applied in Driving under the Influence of Drugs (DUID) monitoring in the U.S.

On-site instrumental analysis in a toxicology context: towards portable capillary electrophoresis for determining gamma-hydroxybutyric acid (GHB) at the point of need

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Background & Aims: The present work was aimed at assembling a novel portable capillary electrophoresis to be used on-site in a forensic and clinical toxicology context for the determination of gamma-hydroxybutyric acid (GHB).

A recent study carried out on 375 urine samples, tested a commercial rapid test for detecting GHB, i.e., Drugcheck GHB single test [1]. According to the authors, the GHB on-site test showed poor performances both in terms of sensitivity and specificity, showing invalid results for vitamin C higher than 10 mg/dL. In the same report, the authors compared the results with an immunoassay test, i.e., the VIVA-E GHB, demonstrating the limitation of detecting exogenous GHB administration in cases of low urinary concentrations [1]. On this basis, an approach based on a separative method for having high sensitivity and specificity is needed.

Undoubtedly, chromatography-based methods represent the most widely used separation approaches, however, implementation in portable equipment is demanding because of the need for large volumes of mobile phases, derivatization reactions for gas chromatography, and a pump in liquid chromatography. Compared to chromatographic separation methods, capillary electrophoresis (CE) demonstrated the ease of making portable instruments that can be used *in situ*. This is because the high voltage power supply used for performing the separations in CE instruments can be easily controlled by an electronic board. On this basis, CE offers several advantages for developing a purpose-made compact device.

Methods: The optimized separation conditions consisted of 20 mM of arginine, 10 mM of maleic acid, 30 µM cetyltrimethylammonium bromide (CTAB) and 5 mM vancomycin (pH 7.35). The separation occurred in a capillary with an internal diameter of 25 µm and a length of 70 cm (total length of 80 cm) applying a voltage of -20 kV. The injection was performed at 0.4 psi x 12.5 sec. The detection was carried out by using a Capacitively-Coupled Contactless Conductivity Detector (C4D) by setting the excitation frequency to 320 kHz and the amplitude to 240 Vpp (peak-to-peak) using a purpose-made amplifier. Alfa iso butyric acid has been used as internal standard at a concentration of 10 µg/mL.

Results & Discussion: A feasibility study for allocating the device inside a box has been carried out. The device is intended to be inserted in a protector case type *Peli1450*® (37.36 cm x 25.96 cm x 15.44 cm). The main parts of the prototype were: pressure controllers, cylinder (300 mL), pneumatic elements, hydraulic components (it integrates a multiport injector with a 10 µL sample loop), Capacitively-Coupled Contactless Conductivity Detector, 2 high-voltage modules, microcontroller board, battery, and safety cage. The preliminary configuration allowed to run 25 analyses, and the performances, of an already validated method [2], have been preliminarily evaluated. The method allowed the determination of GHB within 15 - 200 µg/mL, the limit of detection (S/N = 3) was 4 µg/mL, and the precision of determination was better than 6 %. On this basis, this approach was able to discriminate between low levels of GHB from high levels of GHB, also allowing quantitative results after a proper calibration using spiked real samples. The method was optimized for resolving γ-hydroxybutyric acid from endogenous isomer ß-hydroxybutyric acid (BHB), and a resolution higher than 1.8 was obtained. The method has been optimized for analyzing urine using the dilute-and-shoot approach. After a fourfold dilution, the sample is injected without requiring any derivatization or extraction procedures.

Conclusion: The developed technology can be an additional tool in cases of drug-facilitated sexual assault (DFSA) at the point of need. The use of a rapid on-site instrumental technique will outcome in objective and reliable results that will speed up investigative actions. In addition, this new technology can open up a plethora of further opportunities. The development of suitable equipment for performing on-site analyses which can be effectively used outside of analytical chemistry laboratories for a wide variety of applications and matrices, such as blood, serum, saliva, urine, water, etc. is envisaged.

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DUI O-5

Development, validation, and application of a high-throughput automated sample preparation technique

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Background & Aims: In October of 2023, the Office of the Chief Medical Examiner (OCME) for the City and County of San Francisco, California, implemented an automated liquid handler sample extraction preparation technique of blood and urine for routine use.

The main aim of this project was to translate the existing manual sample preparation method to the automated liquid handler platform, with the further desirable attributes being: increased sample throughput, decreased sample preparation time, and minimized potential for human error.

Methods: In-house method development and programming of the automated liquid handler was performed to ensure the automated technique remains principally similar to the manual extraction method being an acetonitrile protein crash of 0.15 mL of sample, followed by size exclusion filtration, nitrogen dry down and reconstitution with LC starting conditions. Multiple pieces of custom labware were designed, allowing for the exploration of various techniques throughout method development. Labware customization and custom integrations allowed for the two methods to remain nearly identical with changes to consumables differing only to adjust for the requirements of the liquid handler.

Physical limitations associated with automation were also addressed during development. To ensure challenging case samples such as postmortem blood were able to be pipetted, 3.2 mm wide bore tips were utilized and the liquid class settings were optimized. To minimize the risk of cross-contamination, blank physical spaces within the labware (i.e., "travel lanes") were included, ensuring tips do not cross over positions that carry samples. User prompts were introduced to minimize the potential for human error. Lastly, coordinate optimization was performed to ensure correct aspiration and dispensing of all positions.

Results & Discussion: The development of this sample preparation method resulted in an ASB 036 validated automated sample extraction method that leverages the same principles and techniques originally used by the manual sample preparation method. The developed method can prepare 72 samples per batch. Included among the samples is a seven-point calibration curve and quality control samples, all prepared by the automated liquid handler. The typical extraction is completed with two hours of active analyst effort.

Prior to the implementation of travel lanes, trace contamination was observed. Following the implementation of the travel lanes, this challenge has been resolved. Manual intervention is still required following implementation of the automated liquid handler, minimally and where necessary. Specifically, manual intervention is still required to place samples in the size exclusion filtration press, and the nitrogen dry-down steps. While automation is exceptional at performing repetitive, well-defined tasks, there are physical limitations that require manual intervention to ensure intended results. The reduction of user intervention allows for a better-quality product as the potential for human error is minimized. Quality control (QC) sample data from these batches resulted in quantitation within the established Levy-Jennings QC ranges, demonstrating that the automated technique achieved similar or better accuracy and precision to the established manual extraction method. Data logs recording of automated liquid handler actions and pipetting also allowed for expanded tracking and auditing of sample extraction, ensuring efficient root cause analysis when a challenge arises and quality control documentation for court purposes.

As of April 2024, 82 authentic case batches have been prepared using the automated sample preparation technique totaling over 3,000 case blood and urine samples extracted. Over 200 hours of analyst time (25 full working days, over $30,000 in staff resource time) were saved during this period. Further, the implementation of the automated liquid handler allows for one extracting analyst to extract two batches (i.e., 72 samples) in the same time as one manual batch.

The increasing accessibility of automated instrumentation and robotics introduces a unique opportunity to the various disciplines within the field of forensic science. Within forensic toxicology, the implementation of automation allows for the mitigation of human error and maximizes the efficiency of repetitive tasks that do not necessarily require human intervention. The fully streamlined workflow allows for a higher throughput of samples while maintaining robust and reproducible results. The developed method utilizes simple extraction principles allowing for untargeted drug recovery while maintaining effective clean-up prior to sample injection.

Conclusion: The introduction of automation into a forensic laboratory leads to an improved work product through the mitigation of human error. While this is beneficial, it is important to consistently monitor and evaluate the performance of any automation the lab is employing. We have found that in the first year following automation implementation, challenges need to be consistently addressed and the method may require infrequent updates.

Development of a paper-based presumptive test for the detection of fentanyl and its analogues

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Background & Aims: During the past decade, there has been a sharp increase in the illicit use of fentanyl and its analogues. This increase has been attributed to factors such as the opioid epidemic, current shortages of heroin and the overall low cost of fentanyl. From 2019 to 2021 approximately 70,601 deaths were related to the use of synthetic opioids (primarily fentanyl) in the United States. Fentanyl and its analogues are mostly encountered as an adulterant in other commonly used drugs such as methamphetamine and cocaine. Unfortunately, there are a

limited number of presumptive tests available which focus on specifically detecting fentanyl and its analogues. Also, these presumptive tests possess selectivity issues for stimulants and many of these tests face difficulties in providing positive results for fentanyl analogues. Thus, placing great strain on effective forensic detection of drugs of abuse. The aim of this study was to develop a paper based presumptive test for the detection of fentanyl and its analogues, while verifying its potential for in field use.

Methods: To prepare the test strip, Whatman filter paper No44 was cut to size and impregnated with various chemical reagents. The test strips were allowed to dry in a fume hood for 24h before use. Substances including fentanyl analogues were dissolved in acetonitrile and applied to the test strip for observation of a colour change. While developing the test, factors such as buffer environments, test reagent concentrations, dimensions of the test strip and method of analyte deposition onto the test were optimised. Once the test was developed, to deem its usefulness for in field use the following factors were examined. Reproducibility was examined by repeating the test under the developed conditions. Sensitivity was tested via limit of detection or LOD examination. Selectivity testing focused on the rate of true positives and true negatives, using a total of 81 compounds. These compounds consisted of 56 common cutting agents, 13 illicit substances and 12 fentanyl analogues. Stability was examined by allowing the test reagent to age for 3 months and finally the development time of the colour change was determined at different test analyte concentrations.

Results & Discussion: When determining the optimal variables for the presumptive test, it was identified that quinone x (name omitted due to patent application) was the optimal test reagent. This was due to its ability to produce a distinctive blue colour in the presence of fentanyl. To observe a distinct colour change, the test variables required with this compound included a test strip length of 3 cm by 0.5 cm and a phosphate buffer pH 7-8. However, to determine the effectiveness of this test method, the factors for in field use were examined. Firstly, the colour change was reproducible. The LOD of the test strip was identified to be 10 µg however, colour changes (while less distinct) could be visualised at concentrations as low as 3 µg. This is satisfactory as fentanyl is typically seized in quantities ranging from mg to kg. Regarding selectivity, the test method provided a true negative rate of 95% for the total compounds tested, along with a false positive rate of 5%. Also 92% of the fentanyl analogues provided true positive results while 8% provided false negatives. Based on these results, it was identified that the test method was highly selective in the presence of cutting agents and illicit compounds but the presence of fentanyl analogues led to issues in the tests selectivity. It was also identified that aging the test reagent for 3 months did not impact the test method's ability to provide a positive result. Finally, the test method displayed a colour change on average in 2-3 minutes, regardless of the fentanyl concentration used.

Conclusion: This study was able to produce a presumptive test method for the detection of fentanyl and its analogues, which was reproducible, highly sensitive to the presence of fentanyl, highly selective, displayed high levels of stability and provided an overall fast colour change. Based on these factors, this method has the potential for in field use for the detection of fentanyl and its analogues. However, to further validate the tests suitability for real world applications, longer stability times must be examined, and finally more fentanyl analogues and illicit substances must be tested to further verify the selectivity of the test method.

DUI O-7

Prevalence and blood concentration of drugs in fatal road accidents in Western Switzerland from 2002 to 2023

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Background & Aims: According to the last WHO global status report on road safety, road traffic deaths and injuries remain a major global health challenge. In Switzerland, a decrease of road traffic deaths was observed last decades, from 7.0 deaths per 100'000 population in 2002 to 2.6 in 2023. According to the statistics published by the Swiss Federal Statistical Office, 2038 persons died in road accidents in Western Switzerland between 2002 and 2023. Among the cause of traffic accidents, speed, inattention and drugs consumption are the most frequently observed. The Swiss Traffic Law defines legal limits in whole blood to consider a driver irrefragably incapable of driving (0.50 g/kg for ethanol, zero tolerance with technical cut-off for THC (1,5 ng/ml), free morphine, cocaine, MDMA, MDEA, amphetamine, and methamphetamine (15 ng/ml)). To gain more information concerning the type of substances consumed before fatal road accidents and their blood concentrations, we conducted a pioneering analysis of all requests made by the justice in Western Switzerland from 2002 to 2023 regarding this type of event.

Methods: All official DUID cases submitted by the Justice during a period ranging from 2002 to 2023 concerning fatal road accidents as circumstances of death were considered. Post-mortem blood ethanol concentration was determined by HS-GC-FID. General unknown screenings were performed in urine and/or blood by GC-MS. Depending of the substances, GC-MS or LC-MS/MS were used for drugs quantifications in blood.

Results & Discussion: The study included 933 men (83%) and 197 women (17%). The median age of the deceased was 42 (5^{th} percentile: 19; 95^{th} percentile: 82). For 226 cases, only the ethanol quantification was requested, and for 904 cases, a full toxicological analysis was mandated by the justice. At least one psychoactive substance (ethanol, cannabinoids, benzodiazepine, antidepressant, opioid, cocaine, neuroleptic, or amphetamine) was detected in 423 of 904 cases (47%), and more than one substance was detected in 146 of 904 cases (16%). Ethanol was detected in 109 of 226 cases (48%) with only ethanol requested, and 302 of 904 other cases (33%). Median blood alcohol concentration was 1.53 g/kg (5^{th} percentile: 0.15 g/kg; 95^{th} percentile: 2.64 g/kg). Cannabinoids consumption was observed in 108 of 904 cases (12%). Median THC blood concentration was 8.3 ng/ml (5^{th} percentile: 1.6 ng/ml; 95^{th} percentile: 43 ng/ml), and median THCCOOH blood concentration was 18 ng/ml (5^{th} percentile: 4.2 ng/ml; 95^{th} percentile: 54 ng/ml). At least one benzodiazepine or Z-drug was detected in 77 of 904 cases (8.5%). The most frequently detected benzodiazepines were oxazepam (N=26, median: 41 ng/ml), nordiazepam (N=25, median: 170 ng/ml), lorazepam (N=18; median: 20 ng/ml), zolpidem (N=15; median: 51 ng/ml), alprazolam (N=9; median: 33 ng/ml), and bromazepam (N=9; median: 54 ng/ml). At least one antidepressant was detected in 53 of 904 cases (5.9%). The most frequently detected antidepressants were citalopram (N=18, median: 130 ng/ml), mirtazapine (N=8, median: 120 ng/ml), sertraline (N=6, median: 465 ng/ml), and venlafaxine (N=6, median: 420 ng/ml). At least one opioid was detected in 43 of 904 cases (4.8%). The most frequently detected opioids were morphine (N=24, median free morphine: 43 ng/ml), tramadol (N=11, median tramadol: 400 ng/ml; median O-desmethyl-tramadol: 137 ng/ml), codeine (N=10, median free codeine: 18 ng/ml), and methadone (N=10, median: 525 ng/ml). Cocaine consumption was observed in 34 of 904 cases (3.8%). Median cocaine blood concentration was 50 ng/ml (5^{th} percentile: 18 ng/ml; 95^{th} percentile: 320 ng/ml). At least one neuroleptic was observed in 7 of 904 cases (0.8%). The most frequently detected neuroleptics were levomepromazine (N=3, median: 30 ng/ml), and quetiapine (N=2, median: 93 ng/ml). At least on amphetamine was observed in 5 of 904 cases (0.6%). MDMA was detected in 4 cases (median: 668 ng/ml; 5^{th} percentile: 32 ng/ml; 95^{th} percentile: 1385 ng/ml), and amphetamine in 2 cases (median: 44 ng/ml).

Conclusion: The most frequently observed categories of substances in fatal road accidents in Western Switzerland were ethanol, cannabinoids, and benzodiazepines. Central nervous system depressants were more frequently observed than stimulants. For the substances with legal limits defined by the law, medians of concentration were largely above these limits.

Scientific Session 8 – Alcohol and drugs and driving

10:30 – 12:30 Thursday, 5th September, 2024

Chair: Marc Augsburger, Brice Appenzeller

Prevalence of alcohol, drugs of abuse and medicines in fatal road traffic injuries in a Thai population between 2018 and 2023

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Background & Aims: Road traffic injury (RTI) has been a serious problem worldwide. World Health Organisation (WHO) reports that it is the 12th leading cause of death of all ages, and Thailand has the ninth-highest rate of fatalities from that causation. Driving under the influence (DUI) of alcohol and drugs has been well-studied as a risk for RTI. However, the prevalence of DUI of alcohol and drugs in the driving Thai population is currently limited. In addition, the Thai government started approval for medical cannabis policy since 2019 and enacted legislation that allowed cannabis and mitragynine to be legally used in 2020 and 2021, respectively. This may have an impact on drug use trend in Thailand including in RTI. Thus, this study aims to determine the prevalence of alcohol, drugs of abuse, and medication in fatal RTI in Thailand from 2018 to 2023. The trend in drug use in RTI may be altered during this period due to the decriminalization of cannabis and mitragynine. These data will be useful for legal regulations, traffic accident prevention, and forensic analysis in Thailand.

Methods: A retrospective study was conducted on fatal RTI cases that were sent for autopsies at the Department of Forensic Medicine, Faculty of Medicine Siriraj Hospital, between 2018 and 2023. The inclusion criteria were Thai people who were 15 years old or over and died within 3 hours after injuries. All types of RTI cases were recruited including riders or drivers, passengers and pedestrians. The exclusion criteria were cadavers that had signs of

decomposition. The data were collected and separated by the year of death from 2018 to 2023. The data including sex, age, types of RTI, blood alcohol concentration (BAC), and urine drug profiles were recorded for statistical analysis. Urine drug profiles were analysed using liquid chromatography quadrupole Time-of-Flight mass spectrometry (LC-QTOF-MS) and gas chromatography triple quadrupole tandem mass spectrometry (GC-MS/MS). Descriptive statistics including mean, median and standard deviation (SD) were analysed for descriptive data. The comparison between the prevalence of DUI of alcohol and drug use was performed using chi-square test. The comparison of BAC along the period of six years was performed using Kruskal-Wallis H test.

Results & Discussion: From 2018 to 2023, the total number of fatal RTI in the Department of Forensic Medicine, Faculty of Medicine Siriraj Hospital recruited in this study was 1979, divided into 348 (2018), 366 (2019), 316 (2020), 325 (2021), 314 (2022), and 310 (2023), respectively. 1649 cases were male (83.3%), and 330 cases were female (16.7%) and the mean age of all subjects was 37.3 years old. The number of DUI of alcohol who had BAC greater than 50 mg/dL from 2018 to 2023 was 144 (41.4%), 156 (42.6%), 148 (46.8%), 155 (47.7%), 166 (52.9%), and 136 (43.9%), respectively. The mean BAC of each year gradually increased from 86.0, 89.3, 95.3, and 101.9 to 113.1 in 2022 and then declined to 85.9 mg/dL in 2023. The number of DUI of alcohol who had BAC greater than 50 mg/dL and the level of BAC in 2022 was significantly higher than all other years ($p<0.05$) whereas the other five years did not produce any statistical significance.

The number of subjects positive for drugs of abuse and medication in 2018 and 2019 were 43 (12.4%), and 37 (10.1%). However, the number of subjects positive for drugs of abuse and medication increased to 45 (14.2%), 49 (15.1%), 56 (17.8%) and 61 (19.7%) from 2020 to 2023, which showed a significant difference in 2023 ($p<0.05$). The most frequent substances found were methamphetamine (4.8%), followed by cannabis (3.8%), benzodiazepines (3.5%), and mitragynine (3.1%), respectively. Methamphetamine and benzodiazepines did not show significant difference over the period of six years. The number of cannabis use gradually increased along these six years and produced statistical significance in 2023 ($p<0.05$). Mitragynine and ketamine also gradually increased although their figures were not significantly different during these six years. Interestingly, the number of antihistamine and 3,4 methylenedioxy-N-methamphetamine (MDMA) use significantly increased particularly in 2022 and 2023 ($p<0.05$). The most common pattern of drug use in RTI was still single drug use and this pattern was relatively stable along the period of six years. However, the number of multiple drug use (especially more than three drugs) significantly increased particularly in 2022 and 2023 ($p<0.05$).

Conclusion: This study presented a relatively steady trend in the prevalence of DUI of alcohol over the period of six years, except for a significantly higher trend in 2022. Meanwhile, the number of drugs of abuse and medication in RTI markedly increased particularly in 2023. The significant increase of cannabis use in fatal RTI was observed and it was consistent with the legalisation for cannabis in Thailand. In addition, multiple drug use also significantly increased in 2022 and 2023.

DUI O-9

Drugs of abuse detected in oral fluid samples of Victorian drivers over 15 years (2008–2022).

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Background & Aims: Since 2004 Victoria Police have randomly tested the oral fluid of drivers on the roadside for methylamphetamine, methylenedioxymethamphetamine (MDMA) and delta-9-tetrahydrocannabinol (Δ9-THC). From 2008, these specimens have also been screened for up to 40 additional drugs of abuse, including opiates/opioids, stimulants, benzodiazepines, ketamine and cannabinoids. This study was undertaken to determine the prevalence and trends in the detection of these additional drugs in specimens collected as part of the Victorian roadside drug testing program over the last 15 years.

Methods: Oral fluid specimens collected by Victoria Police from drivers at the roadside were transported to the Victorian Institute of Forensic Medicine for confirmatory analysis. All specimens collected between 2008-2022 underwent liquid-liquid extraction before analysis using liquid chromatography tandem mass spectrometry. Positive detections were reported above the cut-off concentrations outlined in the Australian standard AS4760. Data files were collated into batches by year and processed in a single results table for each analyte and related compound.

Results & Discussion: Data from 89,744 oral fluid samples collected from drivers who tested positive for a Victorian Road Safety Act (RSA) proscribed drug between 2008 – 2022 were re-interrogated for additional drugs. Methamphetamine, MDMA and Δ9-THC were detected in 74.8%, 5.2% and 26.0% of cases respectively. The most common drug classes found (excluding the three RSA drugs) were opiates/opioids detected in 9.6% of cases, benzodiaze-

pines in 5.2% and stimulants in 2.7%. A total of 3,745 cases were reported as negative for an RSA drug (4.2% of all cases) while other detected drugs in these cases included cocaine (4.4%), 6-monoacetylmorphine (6-MAM, heroin metabolite, 2.5%), methadone (2.5%) and ketamine (1.9%). Over the study period, 6-MAM was detected in 2.4% of drivers, with 2009 having the highest percentage of cases at 5.0% and 2014 the lowest at 1.6% . Similarly, cocaine, or cocaine metabolites, were detected in 2.5% of cases, with the highest percentage detected in 2009 (5.7%) and the lowest in 2014 (1.1%). After 2014, the percentage of cases positive for cocaine steadily increased until it peaked in 2019 (3.5%). In cases that were MDMA positive but methamphetamine and THC negative, cocaine, or cocaine metabolites, were detected in 23.8% of cases and, similarly, ketamine was detected in 23.5% of these cases. In methamphetamine positive cases that were negative for MDMA and THC, the most common drugs detected were 6-MAM, codeine and morphine, detected in 3.8%, 4.2% and 4.1% of cases respectively. Methadone, and methadone metabolites, were also detected in a large percentage of these cases at 3.1%. At least one benzodiazepine (or benzodiazepine metabolite) were commonly detected in drivers who only tested positive for one proscribed drug, with at least one benzodiazepine detected in 3.8%, 5.1% and 4.3% of cases where only MDMA, methamphetamine or THC were detected respectively, with diazepam and nordiazepam being the most abundant benzodiazepine.

Conclusion: This study provides insights into the drug use behaviours of drivers testing positive to RSA drugs on the roadside and may be of interest to road safety initiatives to understand the prevalence of drugs that may cause driving impairment.

Establishing per se limits in drug impaired driving cases: effects of raising the lower limit for different drugs

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Background & Aims: In Norway, per se limits for drunk and drugged driving are established, corresponding to a blood alcohol concentrations of 0.2 per mille (lower limit), 0.5 per mille and 1.2 per mille. It has recently been questioned from users organizations and in the media that the lower limit for THC (1.3 ng/mL in whole blood) is too low and should be raised to the 0.5 per mille concentration level (3 ng/mL). In this regard, it is interesting to evaluate the level of impairment of drivers who would be able to drive legally unimpaired after such a potential change of the Road Traffic Act. The aim of this study was to investigate the drivers with THC concentrations in the range corresponding to 0.2-0.5 per mille blood alcohol and how many of these that show obvious clinical impairment, as measured by a clinical test of impairment (CTI). We wanted to compare the presence of obvious clinical impairment in this group of THC drivers to drivers where other drugs are detected in legally equivalent concentration ranges.

Methods: All drug impaired driving cases were analyzed for a broad repertoire of ethanol and psychoactive drugs and medicines. Cases were included if only one drug was detected and a valid conclusion of the CTI was available. All drug concentrations were quantified using chromatographic methods and a safety deduction were subtracted from the measured concentrations. The presence of drugs and the drug blood concentrations were compared to the results of the CTI ("not impaired", "mildly impaired", "moderately impaired" or "considerably impaired"). For benzodiazepines, different drugs were converted into diazepam-equivalents. For comparison, a no drug group was included where all analytical results were negative. Information about drug use patterns was not available.

Results & Discussion: A total number of 15 514 individual mono drug-cases was included with the detection of THC (n=6 569), ethanol (n=5 160), amphetamine (n=2 873) and benzodiazepines (n=986). 89% were men and the mean age was 34 years. The number of mono drug cases showing concentrations between 0.2 and 0.5 per mille (or corresponding) was 1 790 for THC, 414 for ethanol, 832 for amphetamine and 134 for benzodiazepines.

For THC, 5.9% of the drivers in this concentration range was assessed as obviously impaired (defined as moderately and considerably impaired) by the CTI, while the comparable numbers were 12% for ethanol, 13% for amphetamine and 9% for benzodiazepines. Among drivers where no drugs were detected, 5% were assessed as obviously impaired.

Conclusion: The present study showed that if the lower per se limit for legal driving was raised from the concentration level corresponding to 0.2 per mille to the level corresponding to 0.5 per mille, 5.9% of the THC positive drivers that would then be able to drive legally was assessed as obviously impaired. This is the same number as among a no drug group. For ethanol, more than twice as many obviously impaired drivers would be able to drive legally if the same change of lower per se limit was made.

DUI O–11

Comparison of the accuracy and precision of THC and cocaine/benzoylecgonine in whole blood when using single point, two point or multipoint calibration in relation to analysis of samples obtained under Section 5A of the Road Traffic Act 1988

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Background & Aims: Driving under the influence of drugs remains a significant problem in the United Kingdom. In order to combat this ever-growing problem, in England and Wales, in March 2015 a new Section 5A of Road Traffic Act 1988 was enacted. This states that it is illegal to drive or attempt to drive with a specified controlled drug in blood above a specified limit. The law is *per se* and a driver that is caught over the specified limit is considered impaired to drive and can be arrested and charged. In England and Wales, there is no further charging related to how much above the limit a driver might be. The law is only concerned with whether the drug concentration is found to be above or below the specified limit. As such, it may not be necessary to accurately determine concentrations either side of the limit. Typically, six or more calibrants are used to create a calibration curve covering the range at which the compound is likely to be detected. Since the Section 5A Act is only concerned with the driver being either above or below the limit, such a large calibration range may be redundant. Therefore, this work investigates whether single or two-point calibration models are comparable to multipoint models in terms of accuracy and precision for quantifying certain Section 5A drugs at the specified limit. The advantage of this approach is a reduction in the method workload and the costs associated with the stock reference materials used to make the calibrants. Furthermore, the single and two point approach negates the need for strict acceptance criteria related to linearity and calibrant outliers when processing an analytical batch.

Methods: Section 5A calibrant and quality control samples at the specified limit for cocaine, benzoylecgonine (BZE) and delta-9-tetrahydrocannabinol (THC) were collected from >100 batches previously analysed using GC-MS/MS (THC) and LC-MS/MS (cocaine and BZE) over the period 2015 – 2023. The original multipoint calibration was then compared to two point calibration with the calibrants bracketing the QC and single point calibration where quantification was based on the straight line from zero to the next highest calibrant after the QC. The QC values, which represent the drugs at their specified limit, were investigated for failures by calculating the mean of duplicate injections and then assessing if each value fell within 20% of the mean, as is done with casework samples. The mean of the duplicate QC injections were then expected to fall within 20% of the expected concentration.

Results & Discussion: For the two point calibration curve, the QCs performed to the same degree of accuracy and precision as the multipoint calibration, as is required for the QC performance for our accredited method. For cocaine the accuracy and precision for the 6-point calibration were 1.8% and 7.3%, for the 2-point calibration were 0.6% and 7.8%, and for the 1-point calibration were 0.2% and 8.7% respectively. For BZE the accuracy and precision for the 6-point calibration were 1.1% and 5.4%, for the 2-point calibration were 1.6% and 7%, and for the 1 point-calibration were 2.6% and 8.6% respectively. For THC the accuracy and precision for the 6-point calibration were 1.3% and 11.7%, for the 2-point calibration were 2.1% and 14.4%, and for the 1-point were 2% and 17.9% respectively. However, for the single point calibration, <10% of QCs were outside of a 20% agreement of the expected concentration of the QC for cocaine, BZE and THC. This is likely due to forcing the line through the origin and dismissing the signal contribution from noise.

Conclusion: Replacing the current multi-point calibration with a 2-point calibration has shown to be successful, allowing for reducing the number of calibration points when testing for Section 5A drugs. This will allow for more samples to be run on an analytical batch and reduce costs.

DUI O–12

The effects of sleepiness on the metabolome – road to biomarkers in oral fluid

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Background & Aims: Nowadays, driving under the influence of sleepiness causes more fatalities than drunk driving. This alarming trend continues despite the integration of a plethora of assistance systems and advanced sensors in modern vehicles. In many cases, drivers' sleepiness is not caused by drugs, resulting in post-hoc negative toxicological reports. By unraveling the complex relationship between sleepiness and the metabolome, forensic toxicolo-

gists can contribute significantly to the development of more precise and targeted methodologies for ascertaining driver impairment. This understanding is essential for establishing a scientific basis to substantiate claims of driver impairment through alterations in relevant endogenous metabolites. Ultimately, a biomarker panel shall be established, allowing the detection of sleepiness in routine forensic analysis.

Methods: For this investigation, we combined the findings of two independent clinical trials. In both exploratory, monocentric, controlled, randomized, and crossover studies, participants underwent different sleep interventions based on commonly occurring real-life sleep/wake scenarios (i.e., sleep restriction, sleep deprivation, and control). In the sleep restriction condition, a sleep deficit of 8 hours (compared to control) was accumulated over four nights, whereas in the sleep deprivation condition, the same amount of sleep deficit was generated by a single night of no sleep. Oral fluid specimens were collected regularly before, during, and after all interventions using Salivette® sampling devices. These samples were screened for metabolites by liquid chromatography coupled to high-resolution mass spectrometry using an untargeted metabolomics approach. In total, 590 oral fluid samples of 30 healthy subjects were analyzed using both reversed-phase (RP) and hydrophilic interaction chromatography (HILIC) and in both positive (+) and negative (-) ionization modes. Raw data underwent batch effect correction (nPYc Toolbox package) and sample normalization steps (probabilistic quotient normalization and auto-scaling) to create a metabolic feature table. A subset of relevant metabolites was compiled by following the rationale that sleep-dependent effects on the metabolome must recede after recovery sleep. Statistical analysis comprised repeated-measures two-factor analysis of variance (ANOVA) and subsequent multiple t-tests between the different study conditions. Significantly altered metabolic features (MF) were tentatively identified via an in-house reference standard library and with the aid of in silico tools (SIRIUS, CANOPUS, CSI:FingerID) where possible.

Results & Discussion: A knowledge-based filtering approach allowed us to flag and exclude MF that either did not follow adaptive sleep-wake regulation or were not robust in nature. With the given workflow, we could monitor 2307 metabolic features (RP +/- 695/70, HILIC +/- 1103/439) that remained for subsequent statistical analysis after filtering procedures. The statistical testing resulted in 231 metabolic features (10.0 % of all detected) with significant alterations at one or more time points after a night of sleep deprivation when compared to 8 hours of sleep. Among these, eight MF were found to be significant at two different time points. Notably, the majority (76 %, 176 out of 231) of the significant MF were detected in HILIC chromatography, underlining the predominantly polar properties of the salivary metabolome. A key finding was that most metabolites were significantly altered when samples were collected in the morning hours (here at 08:10 am). For some metabolites known to be linked with sleep-wake regulation, ANOVA results revealed that the factor 'time' (i.e., sampling time point) was the largest influencing factor (median 9.24 %, range 3.02-42.91 %), even surpassing the intervention factor of sleep deprivation (median 1.81 %, range 0.19-12.46 %). This behavior was frequently observed in metabolites that exhibited a diurnal rhythm under control conditions (morning trough) but showed an irregular pattern following sleep deprivation. However, we observed no evidence of a linear relationship between time since wake and abundance of single metabolites.

Conclusion: Our findings suggest that the effects of inadequate sleep on the human metabolome can be assessed in oral fluid and may be most pronounced in the morning hours, depending on individual circadian rhythm. Importantly, the observed changes are related to the lack of sleep, as they did not occur either before sleep deprivation or after recovery sleep. However, due to the strong influence of time of day on the metabolome, individual inner body time has to be taken into account. It should further be clear that sleepiness cannot be monitored based on a single metabolite but rather has to be assessed based on various relevant metabolite ratios (i.e., metabolic shift). This investigation paves the way for establishing a metabolomic biomarker panel for the identification of fatigued individuals. Unambiguous identification of relevant metabolites will, however, be key for future validation studies in real-world samples and the final application in routine forensic laboratory analysis.

Effects of sampling & handling practices on preanalytical loss of nitrous oxide from whole blood samples

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Background & Aims: Driving under the influence of nitrous oxide is a potential threat to road traffic safety. In Denmark, we have analyzed whole blood samples from traffic cases for nitrous oxide (N_2O) since 2020. The current headspace gas chromatography-mass spectrometry (HS-GC-MS) method remains semi-quantitative, as the volatile nature of N_2O complicates the quantification. Sampling and laboratory handling practices have been suspected of affecting the results, contributing to preanalytical loss of the analyte. We investigated the effects of sample tube headspace-to-fluid ratios and opening of sample tubes prior to N_2O analysis to improve our understanding of the requirements for proper laboratory handling when working with nitrous oxide and/or other very volatile drugs of abuse.

Methods: Blood samples were prepared in 5 mL (13x100 mm) grey-top Vacutainer® tubes to 6 different volumes (5.5, 5.0, 4.5, 4.0, 3.5, and 3.0 mL), spiked with N_2O to a concentration of 8.0 mL/L and analyzed in triplicate and compared to a freshly prepared 8.0 mL/L sample. Another set of triplicate samples spiked to 8.0 mL/L N_2O underwent re-sampling and analysis every day for 3 days. Characteristics of blood samples received to our laboratory in 2023 was investigated. The use of manual gastight syringes was compared to the use of a semi-automated digital syringe for volumetric dispensing of calibrators and internal standards.

Results & Discussion: Recovery of the N_2O from the samples with varying blood volumes were 44.4% (5.5 mL), 38.3% (5.0 mL), 36.5% (4.5 mL), 35.7% (4.0 mL), 31.2% (3.5 mL), and 24.1% (3.0 mL). Samples that had been opened and sampled once and twice contained less analyte compared to those that had not been sampled before N_2O analysis. Authentic blood sample volumes were within 3.4-5.0 mL for 95% of all blood samples received at our laboratory in 2023. (preliminary results)

Conclusion: We found that all headspace-to-fluid ratios investigated had major losses of N_2O as part of the sample preparation. The ratio of headspace to fluid in the sample tube had the biggest effect on the measured concentration of N_2O. We likewise found a significant loss from sampling material from the vials before the N_2O analysis. These findings may provide a framework for proper sampling and handling procedures in laboratories working with volatile compound analysis in biological samples.

DUI O–14

Driving under influence in Finland: Trends and remarks over the past ten years

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Background & Aims: In Finland, a person driving with blood alcohol of at least 0.5 ‰ (g/kg), or breath alcohol of at least 0.22 mg/l, is sentenced for driving under intoxication. Furthermore, with blood alcohol level of at least 1.2 ‰ (g/kg), or breath alcohol of at least 0.53 mg/l a person is sentenced for aggravated drunken driving. Similarly, if driver's blood contains drugs under the Narcotics Act, zero tolerance is applied. Impairment is evaluated if a person has right to use substances under the Narcotics Act (e.g. benzodiazepines), or a person's driving performance may have been impaired by any other drug (Criminal Code, Chapter 23, Section 3). If person's driving performance is significantly impaired, a person can be, if the circumstances are such that the offence is conducive to endangering the safety of another person, sentenced for driving while seriously intoxicated (Criminal Code, Chapter 23, Section 4).

The legislation, as well as modus operandi, are uniform across Mainland Finland. Furthermore, the toxicological alcohol and drug analyses performed as part of driving under influence (DUI) investigations, are nationally centralized and performed in a standardized manner, providing a comprehensive insight to DUI situation in Finland. The aim of this study was to investigate how alcohol and drug use have changed in Finnish driver population during the past ten years.

Methods: All the analyses in whole blood samples were performed at the Forensic Toxicology laboratory under Finnish Institute for Health and Welfare (THL). Alcohol analyses were performed with HS-GC-FID. Drug analyses were performed as comprehensive screening-quantitation of around 80 psychoactive drugs of abuse and medicinal drugs with parallel mass spectrometric techniques, using GC-MS and UHPLC-MS/MS. All the methods are ISO 17025 accredited.

Concentrations of exhaled alcohol levels were measured with evidential breath testing devices placed in police stations and police vehicles.

Statistics used in this study were obtained from databases of the National Bureau of Investigation and POLSTAT (exhaled alcohol).

Results & Discussion: During the past ten years, number of alcohol investigations have continued to decrease, which is consistent with decreasing alcohol use in Finnish general population. In contrast, the number of driving under influence of drug (DUID) cases showed an increasing overall trend. However, after having peaked in 2020, number of DUID cases has returned to a lower curve. Slightly more than 10 % of the cases contained both alcohol and drug analysis requests.

Positive DUID cases contained three drugs on average, indicating that multidrug use is very common. Amphetamine is the most abundant drug finding in Finnish driver population, with proportion of 45–65 % of all DUID cases. The peak year of amphetamine findings was 2020, after which also amphetamine, similarly with total number of cases, has returned to a lower curve. However, amphetamine use peaked in 2020 also in population-level wastewater analyses, indicating that record-high number of amphetamine findings cannot be, at least solely, explained by increased police activity.

The occurrence of cocaine has been steadily increasing, although in the driver population it is still at a low level as compared to amphetamine. It must also be noted that we have not included cases with cocaine metabolite benzoylecgonine only, since there has been Supreme Court decision that inactive metabolites as the only finding do not lead to driving while intoxicated sentence.

Cannabis use has been rather steady with slight increasing trend over the time. Similarly with cocaine, cases containing inactive metabolite (THC-COOH) only, are not included.

Synthetic cathinones have gained popularity during the last few years. First of the noteworthy cathinones was MDPV, the use of which had already diminished by 2013. Alpha-PVP has been the most popular synthetic cathinone during the ten years, with marked increase in positive cases during the last few years. In recent years, two new cathinones have appeared at significant numbers among DUID: alpha-PHP and alpha-PiHP.

Benzodiazepines are still the most encountered medicinal drugs, although their proportion has declined lately. While the number of diazepam positive cases has decreased, clonazepam and alprazolam have gained more popularity. A plausible explanation for this is street trading of smuggled Rivotril (clonazepam) and Ksalol (alprazolam) tablets.

Buprenorphine is the most encountered opioid drug, with a slightly decreasing relative proportion of positive cases. The use of oxycodone has slightly increased.

Conclusion: The long-term increase in number of DUID cases and positive drug findings is consistent with growth in population-level drug use as shown by other indicators such as wastewater-based epidemiology. However, during the last years, the growth in number of DUID cases seems to have stalled. In addition to traditional drugs of abuse, new substances may gain popularity, as exemplified by synthetic cathinones. This emphasizes the importance of keeping up with changes in drug market, and inclusion of new relevant substances in the screened drugs.

Soft mobility and DUI-related traffic accidents: the results of a study conducted in Western Veneto in the years 2019-2023

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Background & Aims: According to the World Health Organization (WHO) traffic accidents are becoming the fifth leading cause of premature mortality worldwide by 2030, mainly because of the increases in motorization. In this context, many countries, including Italy, to persuade people to reduce the use of their car, implemented policies to promote the so-called *soft mobility*, which refers to pedestrian, cycling and, more recently, scooter mobility.

Since the 1960s, numerous studies have proven the role that alcohol and narcotic and/or psychotropic substances play in traffic accidents. However, most of the studies are focused on motorized vehicles (cars and motorbikes). Much less evidence is available about the DUI-related road accidents involving non-motorized vehicles.

The objective of the present work was to present and discuss the toxicological data from road accident cases occurred in Western Veneto in the period 2019-2023. A specific focus was dedicated to accidents involving pedestrians and non-motor vehicles, such as bicycles and scooters.

The work was partly funded by the Department for Anti-drug Policies of the Presidency of the Council of Ministers (PISAD project).

Methods: Blood samples were mandatorily collected from injured people who were admitted to the Emergency Health Care Unit of the hospitals of Western Veneto between January 2019 and November 2023, after they had been involved in a traffic accident.

Blood samples were stored at +4°C until the analysis, which was performed within three days after sample collection at the Laboratory of Forensic Toxicology of the University of Verona.

All the samples underwent toxicological analysis for blood alcohol concentration (BAC) determination and for the search of the most common licit and illicit psychotropic drugs (opiates, cocaine, cannabinoids, amphetamine and methamphetamine, methadone, benzodiazepines and barbiturates)

BAC determination was carried out using a validated head space–gas chromatography–flame ionization detector (HS-GC-FID) method. The analysis of psychotropic drugs was performed by a commercial instrumental immunoassay confirming the positive results by validated LC-MS and GC-MS methods.

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Results & Discussion: A total of 8,905 cases came to attention of the laboratory in the period 2019–2023 (male= 6763; 76%; females= 2139; 24%; median age= 43 y).

Of these 8,905 cases, 17.4% (n= 1,554) showed a BAC > 0.5 g/L (Italian legal limit to drive) and almost half of these (n=847; 46%) showed a BAC > 1.50 g/L. In addition, 17,6% of the examined subjects (n=1564) tested positive for one or more psychotropic drugs, mainly cannabinoids (42%), cocaine (26%) and benzodiazepines (22%).

About 11% of the 8,905 cases (n=956) involved pedestrians, bicycles or motorized scooters.

Among the pedestrians (n=160; 63.1% males; 36.9% females; median age=59 y), 16.3% showed BAC > 0.5 g/L, while 15.6% tested positive for psychotropic drugs.

These percentages were slightly lower for the cyclists (n=722; 78.1% males; 63.1% females; median age=48 y) who showed a BAC > 0.5 g/L in 11.6% of cases and tested positive for drugs in 13.4%, but, dramatically much higher for the scooter drivers (n=74; 90.5% males; 9.5% females; median age=31 y) where almost one third of subjects (27%) showed a BAC >0.5 g/L and almost one fourth (23%) tested positive for drugs.

Conclusion: The present work has shown that a high percentage of road accidents involving *soft mobility* occurs in conditions of alcohol or substance intoxication. Focusing attention on accidents involving scooters, this percentage is significantly higher than that of the general case study (27% vs 17% for drunk driving and 23% vs 18% for drinking under the influence of substances).

The dramatic results concerning the injured scooter drivers could depend on an underestimation of the DUI risk, also given the average age of the subjects (31 years). However, it is also possible that the awareness of being intoxicated leads people to use a scooter instead of a car in the mistaken idea that the rules of the Highway Code do not apply to driving such vehicles. In any case, the intrinsic instability of this type of vehicle significantly increases the risk of accident when driving in a state of intoxication.

DUI O–16

Changing gears in 2023 – putting the brakes on drugs in driving in New Zealand

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Background & Aims: New Zealand's number of fatalities and serious injuries from road crashes is persistently and stubbornly high. In 2022 there were 374 fatalities – an incidence of 7.3 deaths per 100,000 persons. This is the seventh worst of the 39 participating nations in the International Traffic Safety Data and Analysis group (IRTAD). The number of fatalities has persisted across the past decade, spiking at 378 deaths in 2017 and 2018.

The New Zealand government has acknowledged the number of fatalities and serious injuries must reduce and its Transport Agency – Waka Kotahi – has implemented a wide-ranging strategy document. "Road to Zero" was released in December 2019 and seeks a 40% reduction in death and serious injuries (from 2018 levels) by 2030.

The strategy targets multiple areas, including road user choices. 'Road user choices' aims to encourage safer choices and behaviour on our roads. The Institute of Environmental Science and Research Ltd's (ESR) forensic toxicology group provides analytical and technical expertise, as well as research, to support part of this strategy.

ESR is the only laboratory in New Zealand to carry out Land Transport Act *per se* blood alcohol analysis and drug confirmatory analysis. A drug driver regime introduced in 2009 was based on driver 'type' and Class of the drug detected.

Recent New Zealand driver studies have demonstrated the significant involvement of alcohol and other drugs in fatal crashes, for example 48% of fatal crashes in 2022 involved alcohol and/or drugs. The consistently strong relationship between drug use and crashes led to public and political groundswell for roadside oral fluid testing and a more comprehensive drug testing regime, in order to disincentivise drug driving.

Methods: The New Zealand Government formed an advisory panel of five experts, who reviewed published scientific literature, considered New Zealand data from drug-related car crashes and examined drug-driving limits set out

by other jurisdictions. The experts provided a raft of recommendations which included statutory *per se* limits for a limited range of drugs. This proposal was enacted and commenced in March 2023.

Transparency and collaboration were key in this significant legal and process change. First, the ease of access for all to both road crash data and the Expert Panels recommendations enabled buy-in from many. Second, the experts made it quite clear that unlike alcohol, there is insufficient data to associate concentration levels for a specific drug in blood to an effect on driving. This 'front-footing' may have affected the number of court-based challenges to the reported results for this new system. Third, NZ Police and ESR collaborated to create a testing regime based on 'incident type and severity', acknowledged the limitations of some scientific analysis and provided a cost-effective and quick blood testing process, with reports in suitable format for Police Officer ease of use in court proceedings.

In New Zealand there are two key blood alcohol levels for drivers over 20 years. There is an infringement level (50 mg/dL) and an offence level (80 mg/dL). A similar two-tier system of drug levels has been implemented. For 25 drugs there are 'Tolerance' blood drug levels for infringements and 'High-risk' blood drug levels for offences. These 25 drugs include those that are highly prevalent in New Zealand society (such as methamphetamine and cannabis), alongside a range of opioids and sedatives. The presence of other potentially impairing drugs can be reported, and still is.

Results & Discussion: In April 2024 we will complete analysis of the samples submitted during the first full year of this new regime. This presentation will examine the cases submitted and reported, and provide insights into driver type, crash type, drug use and geographical trends.

Preliminary results from the 1750 cases submitted during the first year demonstrate that cannabis and methamphetamine remain dominant in drug-driver samples. Nearly two-thirds of the samples were submitted from hospitalised drivers.

37% of blood samples contain tetrahydrocannabinol (THC) (level range 0.5-61 ng/mL) and 29% contain methamphetamine (level range 10-1300 ng/mL). Other regularly detected compounds include diazepam (3%), tramadol (3%) and methadone (2%). 25% of cases had no drugs detected – nearly all of these were from hospitalised drivers.

Poly-drug use is prevalent with 26% of positive cases containing at least two non-medically administered drugs and several cases containing five drugs with reportable levels. The combined use of methamphetamine and THC is most common. Methamphetamine is also regularly detected with either diazepam and zopiclone.

Probably due to its geographic isolation, New Zealand has a unique illicit drug profile. The detection of three cocaine cases in one year is unprecedented, with benzoylecgonine detected in a further 12 cases. Many novel compounds were also reported including flubromazolam, ADB-BINACA and dimethylpentylone.

Conclusion: It is timely to examine the first year of data to determine what in-roads have been made into New Zealand drug-driving and how the information collected can be used to further improve our justice system and road safety outcomes.

Scientific Session 9 – Clinical toxicology

14:00 – 16:00 Thursday, 5th September, 2024

Chair: Hans Maurer, Naren Gunja

Characterization of the alcohol biomarker phosphatidylethanol in donor whole blood and apheresis red blood cells

CT O-1

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Background & Aims: Phosphatidylethanol (PEth) is a long-term marker of alcohol consumption used frequently in clinical scenarios such as liver transplant evaluation and behavioral medicine. Consensus interpretations for PEth concentrations are: <20 ng/mL (0.03 µmol/L), 'compatible with abstinence or low alcohol consumption'; 20-200 ng/mL (0.03-0.28 µmol/L), 'alcohol consumption'; ≥200 ng/mL (≥0.28 µmol/L), 'strongly suggestive of chronic excessive alcohol consumption'. Recent cases have demonstrated that packed red blood cell (pRBC) transfusion creates the potential for artificial elevation or decrease of observed PEth concentrations in recipients, which confounds interpretation of recipient PEth concentrations, particularly when assessing alcohol abstinence. Currently, very little is known about the prevalence or stability of PEth in blood products. The aims of this study were to characterize PEth concentrations in healthy blood donor populations, and in the corresponding pRBC units from whole blood and apheresis donations.

Methods: Apheresis and whole blood donations were tested for PEth using liquid chromatography – tandem mass spectrometry (LC-MS/MS). Briefly, the 16:0/18:1 (POPEth) and 16:0/18:2 (PLPEth) isoforms were extracted from whole blood using supported liquid extraction (Chem Elute S, Agilent). Calibrators (10-1000 ng/mL) and controls (15, 200, 800 ng/mL) were spiked fresh for each batch. POPEth-d5 and PLPEth-d5 (both 50 ng/mL) were used as internal standards. Extracts were injected onto a Sciex 6500 LC-MS/MS using electrospray ionization in negative ion mode. The assay was validated in accordance with Clinical Laboratory Improvement Amendments standards for Ethylenediaminetetraacetic acid (EDTA) whole blood, with a lower limit of quantitation of 10 ng/mL for both PEth isoforms.

EDTA blood collected at the start of donation in Rochester MN (northern USA), and paired samples from RBC, apheresis-derived (RBC-A) and RBC, whole-blood-derived (RBC-WBD) units were obtained. RBC units were sampled after collection in routine anticoagulant and additive solutions. Additional units donated in Maricopa County, AZ (southwestern USA) were sampled to test PEth stability and the impact of irradiation. Units or pRBC segments were stored under routine blood bank conditions prior to testing to simulate real-world conditions.

This study was deemed exempt from human protections review.

Results & Discussion: Over 40% of apheresis and whole blood donors (n=102) had POPEth ≥10 ng/mL (maximum observed PEth: 587 ng/mL). Of these, 19 donors had POPEth concentrations between 10-20 ng/mL; 48 had POPEth between 20-200 ng/mL; 13 had POPEth >200 ng/mL. As whole blood units were processed into component pRBCs, PEth concentrations increased and were higher than donor whole blood levels prior to collection (maximum observed POPEth: 711 ng/mL). Storage for up to 5 weeks post donation resulted in a mean 17.3% decrease in PEth-positive units; in contrast to a prior report, we observed no PEth formation in units with negative (<10 ng/mL) baseline concentrations. Irradiation of pRBCs did not substantially affect PEth concentrations or stability in either PEth-positive or PEth-negative units.

PEth prevalence and concentrations in healthy blood donors are sufficient to potentially confound alcohol use or abstinence assessment in pRBC recipients. Although there was some relationship between EDTA PEth concentration and the measured PEth in pRBC units, donor PEth was only loosely correlated to the PEth measured in blood products. Processing to pRBCs tended to increase the observed PEth concentration, but there was substantial variability, particularly from whole blood donations.

Conclusion: Currently, it is unknown how long PEth may persist to detectable levels following a transfusion; more studies need to be performed to provide recommendations since the detection window will be dependent on the volume of PEth positive units transfused and any on-going patient blood loss. pRBC transfusion has the potential to cause either false positive or false negative PEth results in blood product recipients. Toxicology, transfusion medicine and clinical practices such as transplantation and behavioral medicine should recognize this phenomenon and collaborate on testing protocols to appropriately interpret PEth in pRBC recipients.

CT O-2

Ayurvedic medicine: A 'natural' way of managing type 2 diabetes? A case study

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Background & Aims: Diabetes is a chronic illness characterised by elevated levels of blood glucose. Commonly prescribed diabetes medication includes metformin and sulphonylureas (glimepiride, gliclazide, glipizide and glyburide). The main goal of these oral medications is to help lower blood glucose levels to within a health range. These medications can increase the risk of hypoglycaemia and have several potential side effects, both minor and severe.

Ayurveda is a system of traditional medicine native to India that relies on a natural and holistic approach to physical and mental health. There are a few studies that suggest that Ayurvedic medicine might help manage symptoms in people with type 2 diabetes, however most of these trials are small or not well-designed.

In this case study a doctor at a diabetic clinic was approached by a husband and wife (late 50's) with established type 2 diabetes. The couple wanted to treat their diabetes 'naturally' and had concerns about taking Western medication. They had been managing their diabetes with Ayurvedic medicine prescribed by a doctor and obtained from a local health pharmacy. Before agreeing to treat the couple, the doctor at the diabetic clinic wanted to test the Ayurvedic medication for the presence of any active hypoglycaemics. The aim of this study was to analyse the pills being taken by the couple in order to determine if they contained any active diabetic medication. The wife's Ayurvedic regimen included five pills and three powders, and the husband was taking four pills, all made up of different preparations.

Methods: In total nine pills and three powders were received for analysis. Each of the pills was crushed into a powder. Water (2 mL) and acetonitrile (2 mL) were added to separate Eppendorf tubes containing the crushed pills and

powder samples. These were vortex mixed and sonicated. The samples did not fully dissolve, and after the sediment had settled, 10 µL of each pill solution was added to 990 µL injection solution (water: methanol, 9:1, v/v). 5 µL was injected into the SCIEX X500R QTOF system.

The vMethod application (designed by SCIEX) was used for the analysis of the pills. Separation was achieved using a Kinetex 2.6 um Phenyl Hexyl (50 mm x 4.6 mm) column with 10 mM ammonium formate in water (mobile phase A) and 0.05% formic acid in methanol (mobile phase B) at a flow rate of 700 µL/min. Positive electrospray ionization was used for data acquisition in Sequential Window Acquisition of All Theoretical Fragment-Ion Spectra (SWATH) mode. All mass spectra were acquired from *m/z* 50 to 900. The instrument was automatically recalibrated every five sample injections using ESI positive calibration solution delivered through a calibration delivery system. SCIEX OS v3.1.6 software was used to control the instrument and for the processing of the data. Compounds were identified by MS/MS library matching with a SCIEX Forensics High-Resolution MS/MS spectral library. The confidence criteria used for compound identification were mass error, retention time error, isotope ratio difference and library score.

Results & Discussion: All of the medication being taken by the wife contained varying amounts of the diabetes medication, metformin. Additionally, one of the sulphonylureas, glyburide, was found in two of the pills. Two out of the four pills being taken by the husband contained metformin. No drugs were detected in the third pill, however in the fourth pill, strychnine was detected. Strychnine is obtained from the seeds of *Strychnos* nuxvomica, a tree native to India, and is (today) used in the United States, primarily as a pesticide to control rodents.

These results show that uncontrolled amounts of diabetic medicines were added to the Ayurvedic medicine regimen prescribed to these patients. The amount of metformin and glyburide added to the pills was not regulated. Not only does this make the patients more susceptible to experiencing side effects of the medication, this can also cause several complications in the management of the patients' diabetes.

Unfortunately, even after being presented with the results from the analysis of the pills, the patients chose to continue taking the Ayurvedic medication for the management of their diabetes and were going to rather follow up with a 'natural' doctor.

Conclusion: In conclusion, these two patients who initially chose to treat their diabetes naturally through Ayurvedic medicine to avoid potential side effects of Western medication ended up, unknowingly at first, taking unregulated amounts of Western diabetic medication. These results were forwarded to the South African Health Products Regulatory Authority (SAHPRA) which is a public entity responsible for the regulation of health products intended for human use. The results from the analysis of samples in cases such as this are important and if reported to the relevant regulatory authorities have the potential to protect patients from unknowingly taking medication that is not regulated.

Accidental intoxication of three policemen with ADB-BINACA after inhaling substance containing dust

CT O-3

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Background & Aims: The usage of synthetic cannabinoid receptor agonists (SCRAs) in younger population is known for recent years. But not only the consumption also the production of SCRA containing products like coated CBD-rich or even THC-containing cannabis-products and so-called c-liquids (SCRA-containing e-liquids) could lead to serve intoxications. However, we describe three intoxications of police men after accidental high dose contact with ADB-BINACA (N-[1-(aminocarbonyl)-2,2-dimethylpropyl]-1-(phenylmethyl)-1H-indazole-3-carboxamide) while investigating a potential cannabis plantation. While investigations it turns out to be also a production place of SCRA containing cannabis products and vapes. After deinstallation of an exhaust fan in an unused attic, the policemen where exposed to a cloud of ADB-BINACA containing dust. The policemen were hospitalized, and serum samples were taken for further examinations. Additionally seized samples also those taken from the exhaust fan were analyzed in the lab of the state office of criminal investigation.

Methods: Serum samples were analyzed for classic drugs of abuse in the local hospital and in the authors lab using immunoassays, gas-chromatography-mass spectrometry (GC-MS) and liquid chromatography coupled with a quadropoly time-of-flight mass spectrometry (LC-QTOF) screening methods after solid phase extraction (SPE) on a C18-column for sample preparation. While quantification of ADB-BINACA and determination of metabolites was not possible for serum samples in the authors lab, a specialized external lab (Department of Forensic Toxicology, University of Freiburg, Germany) was commissioned. Seized cannabis products were qualitatively analyzed after ethanolic extraction using a GC-MS while GC coupled with a flame ionization detector (GC-FID) was used for quan-

tification of containing THC. SCRAs were identified using GC-MS screening and LC coupled with ion-trap (ToxTyper®) after extraction using chloroform/methanol (v:v, 1:1). Particle size of the identified ADB-BINACA powder was determined by using scanning electron microscope coupled with energy dispersive X-ray spectroscopy (SEM-EDX).

Results & Discussion: The seized cannabis materials were mainly buds, haze, and extracts containing ether higher CBD-concentrations or Δ7-, Δ8-, Δ9-THC-concentrations. The analyzed powder contains high concentrations of ADB-BINACA with determined particle size ≤1 µm in relevant amounts. Due to particle size and high concentration intoxication by inhalation was generally possible. Screenings for classic drugs of abuse and other medication in the taken serum samples were negative. Serum concentrations of the three policemen for ADB-BINACA were 1.4, 3.1, and more than 10 ng/mL. Those policemen were between 37 and 57 years old, physically fit, healthy with no long-term medication. They often had to handle those kinds of situations and were well trained. However, they were wearing hand gloves but no face masks for any reason. After contact with the ADB-BINACA containing dust onset of symptoms began after approximately 10 minutes and were described as follows: tachycardia (up to 166 bpm), hypertension (up to 200/100 mmHg), dyspnea, disorientation, extensive sweating, and the feeling of losing touch with reality as well as fear of death. They had to rest on the grass outside, sitting or standing was physically not possible. The officer with the highest measured serum concentration of more than 10 ng/mL ADB-BINACA (57 y.o.) also described loss of memory and had to be treated with i.v. metoprolol but finally fully recovered and was fit for service after a couple of weeks. The officer with the medium serum concentration of 3.1 ng/mL (37 y.o.) fully recovered and was fit for service within 5 days. He only described kind of hangover for 2-3 days after hospitalization. The 42 y.o. man with the lowest determined serum concentration of 1.4 ng/mL ADB-BINACA showed the mildest physically symptoms but the worst psychological symptoms as episodes of depersonalization, panic attacks, flashbacks, and lack of concentration. A post-traumatic stress disorder (PTSD) was attested, and he finally was fit for service after more than 5 months but psychological not fully recovered yet. However, other officers also standing in the attic with more distance to the dust were not notable affected.

Conclusion: This case describes an alternative accidental way of intoxication with ADB-BINACA after inhaling of SCRA containing dust by three policemen. The high concentrated dust with the low particle size led to serve intoxication symptoms after inhalation. The physically and psychological symptoms showed no correlation with the different determined serum concentrations of 1.4. – 10 ng/mL. Also, long term disorders like PTSD were possible and also associated with lower serum concentrations. And finally, it shows the importance of careful handling unknown substances in each situation.

CT O-4

A case of acute psychosis post consumption of Delta-9-o THC and HHC laced edibles.

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Background & Aims: In recent years, synthetic cannabinoids have surged in popularity as substitutes for traditional marijuana products. Among these alternatives are compounds like HHC (Hexahydrocannabinol) and Delta-9-o THC (Delta-9 tetrahydrocannabinol acetate, also known as Delta-9o, D9o or Delta-9 THCo) which offer users a potent experience akin to natural cannabinoids but with potentially heightened risks. As the market for synthetic cannabinoids continues to expand, driven by factors such as accessibility and perceived legality, concerns regarding their unregulated nature and unknown pharmacological effects are largely ignored. Children and young adults are especially vulnerable and easily deceived by the product labeling and legal claims, subjecting themselves to harm. While traditional marijuana has been extensively studied, the physiological impact of synthetic counterparts such as Delta-9-o and HHC remains largely unknown.

This investigation aims to address the critical gap in understanding of these synthetic cannabinoids by focusing on a hospitalization case in the United States following ingestion of gummies laced with HHC and Delta-9-o THC. By analyzing this case in detail, including the user's understanding of products, clinical presentations, laboratory findings, and treatment outcomes, the aim is to shed light on the specific risks associated with these emerging synthetic cannabinoids. By engaging the impacted population (youth) in this study as co-authors, another critical aim of this work is to leverage peer to peer conversations in educating the most vulnerable group i.e., the youth. Teenage authors in this study will leverage their learnings from this work to educate their peers regarding the harmful effects of these "so-called" legal products. This unique approach is critical as our young adults and the next generation must be cautious and aware of the risks that these drugs entail. Ultimately, this work aims to inform public health efforts and policy initiatives aimed at safeguarding vulnerable populations.

Methods: In-depth interviews were conducted with the user and their two friends to obtain the timeline of the events and to collect information regarding the subjective symptoms from the user's perspective. All three individuals volunteered to provide this information with the goal of educating others. The friends interviewed were present during the incident and took the gummies themselves but did not have any adverse effects. They took the patient to

the hospital and provided care during hospitalization and afterwards. Additionally, use history and product labelling was reviewed and user's understanding of the safety of products was assessed via interview. Hospital records and findings were reviewed, clinical symptoms were documented based on hospital findings and eye-witness reports.

Results & Discussion: Within one hour of ingesting one gummy on the eve of New Year 2024, acute auditory hallucinations were reported by the user leading to severe psychiatric manifestations that lasted more than a week. The individual was hospitalized on day three of the incident by two friends who got increasingly worried about the deteriorating mental condition of their friend. In-hospital toxicology screening was conducted resulting in a positive response on the cannabinoids screen. Confirmation testing was not ordered by the hospital treating the patient and hence the product labeling could not be verified. However, the product label claimed to contain delta-9-o THC, 25mg. Additionally HHC and Delta-9 THC were listed in the ingredients list. The patient was interviewed three weeks after the episode and vividly remembered all details of the two weeks long episode and volunteered all the information regarding their experience. The account of events was verified by the friends present during the incident who took this individual to the hospital. Three other individuals, including the two friends who took the patient to the hospital, had consumed the same product at the same time but did not experience any adverse events. The case history and the associated subjective adverse effects will be discussed in detail during this presentation to inform the audience of the clinical manifestations and treatment outcomes.

Conclusion: The escalating prevalence of synthetic cannabinoids like Delta-9 THC-o and HHC underscores the urgent need for further research to elucidate their safety profile. The case involving adverse reactions, including severe psychiatric manifestations and physiological distress, presented in this report raise red flags about their potential hazards.

Investigating γ-hydroxybutyrate (GHB) detection time and associated symptoms: Insights from a randomized clinical trial

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Background & Aims: γ-hydroxybutyrate (GHB) is important in forensic investigations, due to its potential use to incapacitate victims of crime. However, detecting GHB is challenging due to its rapid elimination and endogenous concentrations. Current guidelines state that GHB intake can be detected for up to 6 hours in blood and up to 12 hours in urine.

As part of a randomized clinical trial aiming to detect biomarkers with metabolomics to prolong the detection time of GHB, we here report simultaneous measurements of GHB in blood, urine, and saliva after controlled intake of GHB, using an optimized UPLC-MS/MS method. Furthermore, we report symptoms experienced by the participants.

Methods: Thirty healthy volunteers were randomly assigned to receive a single dose of GHB (sodium oxybate, 50 mg/kg) or placebo. Blood, urine, and saliva samples were collected at baseline, as well as at 1, 2, 3, 4, 6, and 12 hours after exposure. Additionally, urine samples were collected 24 hours post-exposure. Blood samples were collected in tubes containing a fluoride oxalate mixture. Urine and saliva samples were collected in plastic cups and transferred to tubes without additives. All samples were cooled immediately after collection and transferred to long-time storage at -70 degrees within less than 7 hours. GHB was quantified in all matrices using a sensitive LC-MS/MS method with lower limits of quantification (LLOQ) of 5 ng/mL in blood and 10 ng/mL in urine and saliva.

The participants in the trial rated symptoms (e.g. dizziness, fatigue, headache, and nausea) on a scale of 0 to 10 at each sample collection time point.

Results & Discussion: In the placebo group, median GHB concentrations were 14 (IQR=11-16) ng/mL in blood, 82 (IQR=55-140) ng/mL in urine, and 110 (IQR=63-170) ng/mL in saliva, when considering all measurements across collection times (n=13-15, 7-8 samples pr. participant). No trends in concentrations within and between days were observed. Comparing GHB concentrations in the active treatment group (n=14-15) to those of the placebo samples at the corresponding time point (n=13-15), we found that the GHB concentrations were significantly elevated for 12 hours in blood and urine, and for 6 hours in saliva in the active treatment group. Similarly, the GHB concentrations in the active treatment group were significantly different from baseline for 12 hours in blood and urine, and for 6 hours in saliva.

The maximum median concentrations (C_{max}) observed in the different matrices were 50,000 (IQR=41,000-59,000) ng/mL in blood, 440,000 (IQR=280,000-510,000) ng/mL in urine, and 29,000 (IQR=18,000-48,000) ng/mL in saliva. The time to peak concentration (T_{max}) was consistent across all matrices, occurring 1 hour after administration.

The participants in the active treatment group reported varying degrees of nausea, dizziness, fatigue, confusion, and affected hearing or vision. Furthermore, they experienced difficulties concentrating and a diminished con-

trol of their movements. These symptoms, coupled with a general feeling of being affected by the drug, were most prominent after 1 hour. As such, the highest scores (9 out of 10) registered were after 1 hour to feelings of dizziness, fatigue, and concentration difficulties. Apart from being hungry and slightly tired, only few and mild symptoms were reported in the placebo group.

Conclusion: Utilizing optimized sample collection with immediate cooling and sensitive LC-MS/MS analysis, this study demonstrates higher GHB concentrations in blood and urine for up to 12 hours after intake, and in saliva for up to 6 hours compared to endogenous levels. Moreover, the symptoms reported by the participants following GHB intake, provide valuable insights to the existing forensic understanding of symptoms associated with GHB intake. These findings enhance our understanding of GHB pharmacokinetics and highlight the importance of controlled sample storage and sensitive detection methods in forensic GHB investigations.

CT O-6

Investigating psilacetin metabolism: in vivo conversion to psilocin in male rats

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Background & Aims: 4-Acetoxy-dimethyltryptamine (psilacetin) is a synthetic analog of 4-phosphoryloxy-dimethyltryptamine (psilocybin) which is used recreationally for its psychedelic effects. Psilacetin is a putative prodrug for psilocin, but there is limited evidence for the conversion of psilacetin to psilocin *in vivo*. Thus, the goal of the present study was to determine plasma concentrations of psilacetin and psilocin after administration of psilacetin to male rats, and to relate plasma pharmacokinetic measures to pharmacodynamic effects.

Methods: Male Sprague-Dawley rats (300-400 g) received subcutaneous (s.c.) injection of psilacetin (0.1, 0.3 1.0 or 3.0 mg/kg) or its saline vehicle. The administration via s.c. route in rats is optimal for modeling the p.o. route in humans, especially regarding peak drug concentrations in the bloodstream. Plasma was collected and pharmacodynamic effects (temperature, locomotor score, and wet dog shakes) were determined at 0, 15, 30, 60, 120, and 240 min time points. A total of 180 rat plasma samples were quantitated by a fully validated LC-MS/MS method. Samples were extracted by combining 50 µL of plasma with 50 µL of 0.1 M ascorbic acid and 25 µL of a 0.1 µg/mL internal standard solution, followed by protein precipitation with 250 µL cold acetonitrile. The mixture was vortexed and centrifuged (16,873 rcf for 10min). The supernatant (200 µL) was evaporated at 55°C under nitrogen, and the residue was reconstituted in 100 µL of mobile phase (95:5, 0.1% formic acid in 10 mM ammonium formate (pH 3):0.1% formic acid in acetonitrile). The method used a Kinetex C18 (2.6 µm, 2.1 × 100 mm,) chromatographic column and ESI positive mode with two MRM transitions monitored for identification, with a total run time of 10 min.

Results & Discussion: Pharmacokinetic data demonstrated a dose-proportional relationship between psilacetin dose administered and psilocin plasma concentrations and indicated complete conversion of psilacetin to psilocin *in vivo*. Moreover, Cmax values ranged from 6.1 to 87.7 ng/mL after 0.1 to 3.0 mg/kg doses, respectively. These values closely resemble those observed in human subjects, further indicating the comparability of s.c. administration in rats to the p.o. route in humans, particularly concerning measured Cmax values. Pharmacodynamics revealed a temperature decrease which was significant at higher doses. To the best of our knowledge this is the first *in vivo* study evaluating pharmacokinetics and pharmacodynamics of psilacetin across various doses and time intervals.

Conclusion: This study demonstrates that psilacetin is rapidly converted to psilocin in rats, and the parent compound was not detected, even at the earliest time point post-administration. Psilocin plasma levels in rats receiving psilacetin mirror those observed in humans receiving psilocybin, showing a dose-dependent relationship. Higher psilacetin doses (1 and 3 mg/kg) lowered body temperature. The dosages used in this study are in line with those reported previously. In conclusion, psilacetin exhibits a prodrug mechanism analogous to psilocybin, suggesting psilacetin might serve as a psilocybin-like treatment in humans.

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Use of an untargeted screening workflow to detect new LSD metabolites following controlled LSD administration to humans

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Background & Aims: Lysergic acid diethylamide (LSD) is a semisynthetic classic psychedelic drug derived from lysergic acid, a substance found in the parasitic rye fungus. LSD is one of the most potent psychedelics known, and doses as low as 25 µg can cause alterations in consciousness. LSD poses a substantial analytical challenge due to its instability, the low concentration of detectable parent drug, and extensive metabolism. Its metabolism has been studied in animals and in vitro experiments postulating numerous LSD metabolites, but most of these were never detected in forensic casework. Systematic (untargeted) metabolism studies in human biological matrices are scarce. Therefore, this project aims to adapt an untargeted analytical method and a data analysis workflow commonly used in metabolomics to systematically screen for and identify new LSD metabolites following controlled LSD administration to humans that are more consistently detectable in human urine samples.

Methods: Urine samples of twenty healthy subjects from a placebo-controlled, randomized, cross-over LSD study were utilized (Holze et al, Neuropsychopharmacology, 2022). The urine samples, collected over 8h after the administration of a 200 µg dose of LSD or placebo, were protein precipitated and analyzed using an untargeted liquid chromatography high-resolution mass spectrometric analysis method (qTOF-MS Sciex 6600; reversed phase, ESI+, data-dependent acquisition, mass range m/z 50-1000). Untargeted data was processed using MS-DIAL (version 4.9.2) for peak picking, retention-time alignment, and blank filtering. Statistical testing and filtering were done in R (version 4.3.2). Changes between the placebo and LSD condition were compared individually per participant (paired samples, individual fold-change) and across the entire dataset (median fold-change). The data was subsequently filtered according to the following criteria: linearity in a QC-dilution series (R^2>0.8), median fold-change > 2, p-value < 0.05 (Wilcoxon test), and increase in individual fold-changes in at least 70% of the paired samples. Sirius (version 5.8.6), an in-house database, and manual structure elucidation were used for preliminary identification.

Results & Discussion: Peak picking yielded 20'943 features which were reduced to 57, using the specified filtering criteria. These features represent the most promising LSD biomarkers, given their consistent presence among study participants. The feature with an *m/z* of 356.1956 showed the highest median fold change (670). Sirius identified this feature as LSD's main metabolite 2-oxo-3-hydroxy-LSD, and its retention time, MS1, and MS2 spectra matched the reference standard. This demonstrates the workflow's ability to detect known LSD metabolites. None of the other described known LSD metabolites, such as nor-LSD or hydroxy-LSD were identified within the strictly selected feature list, due to low sensitivity. Hydroxy-LSD glucuronide (*m/z* 516.2340, fold-change infinite) also failed the linearity criteria but was manually identified in 18 samples. Looking at the MS2 spectra of 2-oxo-3-hydroxy LSD and LSD, common fragment ions are either *m/z* 221, *m/z* 222, and/or *m/z* 223. A fragment ion of *m/z* 221 is also present in the MS2 spectra of the selected features with a precursor *m/z* of 295.2051, 363.2151, 379.2114, 382.2591, and 400.2322 pointing to LSD-related structures. Of these, the feature with an *m/z* of 400.2322 showed the second-highest median fold change (80) and was preliminarily identified as a carboxyl derivative of 2-oxo-3-hydroxy LSD. Six other features contained a fragment ion of *m/z* 223. Sirius identified four of these features as cortisol-related compounds, which, combined with an observed fold change of 2-3.5, indicates that these features are likely endogenous compounds. Finally, seven features with two MS2 fragments in common (*m/z* 130 and 84) showed a median fold change greater than five, suggesting an exogenous origin. None of these resembled other expected LSD fragmentation patterns and could not be tentatively identified so far.

Conclusion: Using the adapted workflow, we were able to detect a probable new metabolite of LSD, namely a carboxyl derivative of 2-oxo-3-hydroxy LSD. Several other features showed promising characteristics but have not yet been identified. This may broaden the panel of metabolites actually being detectable in urine samples. Our results demonstrate that the sole examination of common or typical fragments does not guarantee the detection of LSD metabolites or derivatives but that untargeted data filtering may reveal other promising biomarker candidates. A group of endogenous compounds was identified by chance, showing that this workflow can be used for more than finding new drug metabolites. If suitable, these endogenous compounds could be used as indirect biomarkers for LSD use. This could help to study LSD-use patterns or to search for underlying toxicological aspects of LSD-effects in humans. Overall, this workflow shows potential for detecting new metabolites as well as endogenous markers after LSD consumption and could also be applied to other drugs of abuse that share analytical challenges with LSD.

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Two cases of intoxication with delta-9-tetrahydrocannabiphorol (THCP), a new semi-synthetic cannabinoid.

CT O-8

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Background & Aims: Tetrahydrocannabiphorol (THCP) is a potent phytocannabinoid naturally occurring in trace amounts in cannabis. It has a 30- and 5.8-fold greater affinity than THC for CB1 and CB2 receptors, respectively [Bueno J. et al. J Nat Prod 2021;84:531-536], due to its two-carbon longer alkyl chain. The current lack of regulation has led to the marketing of legal products, labelled THCP, claiming effects similar to those of cannabis, as previously for hexahydrocannabinol and its analogues. We report the first cases of delta-9-tetrahydrocannabiphorol (THCP) intoxication following oral consumption of oils in a recreational setting.

Methods: Non-targeted toxicological screening in blood, urine and oils were performed by LC-HRMS (Orbitrap Exploris 120, ThermoFisher Scientific) using a previously published method [Becam et al. J Chromatogr B Analyt Technol Biomed Life Sci 2023;30:1224:123739]. Oils were also analyzed by GC-MS (GC : 7890A, MS : Agilent 5975C, Agilent). The oil labelled THCP was also analyzed by NMR (Bruker Avance III™ HD 500 MHz).

Results & Discussion: During the night of 07/12/2023, a 42-year-old woman and her 36-year-old companion consumed 2 drops of 2 oils: the first labeled cannabidiol (CBD) 4%, and the second THCP 10,000 mg, both purchased in a CBD shop. On the night following ingestion, the first patient experienced nausea, vomiting and dizziness, which led her to consult the emergency department. On arrival, she presented with partially reactive mydriasis and major spastic hypertonia. She progressively deteriorated neurologically, with a Glasgow sc (GCS) = 10, and lactatemia at 3 mmol/L (VN = 0.5 - 1.5 mmol/L). The electroencephalogram showed an encephalopathic profile despite normal brain imaging. She was transferred to a neurology unit, where she was discharged on 12/12/2023 following complete resolution of her neurological disorders. At the same time, her partner was also admitted to the emergency department, presenting with a neurological picture of vigilance disorders (GCS=13), obnubilation, nuchal rigidity, pyramidal syndrome, epileptoid tremor of the lower limbs, fine apraxia and loss of strength. The EEG showed encephalopathic phenomena. The patient was transferred to a psychiatric intensive care unit. She was moody and agitated, and exhibited echolalia with unmotivated laughter, inappropriate singing and abnormal behavior, necessitating the administration of cyamemazine. On 11/12, she presented a psychiatric picture with hypomania and agitation. She was transferred to a psychiatric hospital for in-patient treatment, but was later discharged even though her condition had not fully improved. She was subsequently seen several times for follow-up consultations. Since the poisoning, the patient has suffered from depression and has yet to resume her professional activities.

Following their admission in emergency department, a blood sample was drawn and sent to the toxicology laboratory for analysis along with both oils.

Toxicological screening by LC-HRMS found CBD in CBD oil only (not detected in urine and blood, LOD = 0.5 ng/mL), and other samples were negative. THCP was absent from the libraries used in these reprocessings (mzCloud™, NIST LC-HRMS, HighResNPS). NMR analysis reveals the presence of THCP at a level of 12% in the corresponding oil. Plasma THCP concentrations were 12.1 ng/mL for the first patient and 11.9 ng/mL for the second. It was not detected in urine (LOD = 0.5 ng/mL).

Conclusion: To our knowledge, there are no pharmacological or clinical data on THCP. Nevertheless, the severity of the symptoms experienced by the two victims, probably linked to THCP's high affinity for CB1 and CB2 receptors, calls for great vigilance with regard to this compound. It also seems important to include THCP in toxicological screening.

Glucagon like peptide-1 analogues and misuse for weight-loss purposes: semaglutide and liraglutide proteins analysis in whole blood samples by the use of LC-ESI-HRMS method

CT O-9

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Background & Aims: The use of GLP-1 analogues has been increasing worldwide in recent years due to their benefits in treating type II diabetes. Thanks to their effects on appetite regulation, in many countries they are also used, especially semaglutide, to treat obesity. However, due to their promotion by social media and celebrities as a weight-loss treatment, GLP-1 analogues are misused by a non-diabetic and non-obese population and also by a young public, which is the main target of these media. Following the alert by the ANSM (Agence nationale de sécu-

rité du médicament) in France and the FDA (Food and Drug Administration) in the United States, which imposed the addition of fatal effects to the list of side effects for semaglutide, the misuse seems to become a public health problem. For this reason, it seems important that a toxicology laboratory is able to evidence the presence of these drugs in blood. In this study, the authors have developed and validated a method for the identification and quantification of semaglutide and liraglutide in whole blood using a LC-HRMS method.

Methods: LC separation was achieved using a Waters Acquity HSS C18 column (150 x 2.1 x 1.8 µm) with a controlled temperature maintained at 50 °C. A 5-µL injection with a 0.25 mL/min flow of waters with 0.1 % of formic acid (solvent A) and acetonitrile with 0.1 % of formic acid (solvent B) was used. A Xevo G2-XS Q-TOF high-resolution mass spectrometer (Waters corporation, Milford, MA, USA) was used, operating in positive ion mode and in sensitivity mode. In MS scanning, data were acquired from 500 to 2000 m/z. For semaglutide, the 4-fold charged $[M + 4H]^{4+}$ molecule was observed at m/z 1029.29752 (which deconvolutes to 4117.70139), while for liraglutide the 4-fold charged ion $[M + 4H]^{4+}$ was observed at 938.76490 (which deconvolutes to 3754.53707).

The extraction was performed by blood protein precipitation using a mix of ACN/MeOH (70:30), after the addition of 50 ng/mL of internal standard (bovin insulin). The method was applied to authentic whole blood samples following a hospital request for GLP-1 analogs determination and to postmortem blood samples. The blood samples were stored at + 4°C until analysis.

Results & Discussion: Usual therapeutic blood concentrations of both drugs are in the range 50-150 ng/mL. The validation procedure demonstrated an acceptable linearity between 2 and 500 ng/mL. LOD and LOQ were 1 and 2 ng/mL, respectively. Intra and inter-day precision were below 20 % at three concentrations. The method was successfully applied to the blood samples of 3 diabetic patients under treatment of semaglutide (concentrations ranged from 31 to 70 ng/mL) and to one postmortem blood sample which tested positive for liraglutide at 12 ng/mL. These concentrations fall within the limits of therapeutic blood concentrations described in the literature.

Conclusion: The use of semaglutide and liraglutide is increasing for the treatment of type II diabetes but along with its therapeutic use, its misuse for weight loss is also increasing. Because of their side-effects, which can be lethal, it seems essential to be able to document an intake of these drugs. The method developed aims to highlight a therapeutic administration of semaglutide and liraglutide, to be able to also evidence the presence of traces in case of improper administration and also to ensure a forensic application on whole blood, which is the most common matrix in this field.

Scientific Session 10 – Analytical methods

16:30 – 18:00 Thursday, 5th September, 2024

Chair: Dirk Wissenbach, Simon Elliott

AM O-1

Evaluation of spectroscopic techniques for on-site drug testing on festival seizures

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NICC, Brussels, Belgium

Background & Aims: Belgium has a wide variety of music festivals which are visited by an international scene. Despite the fact that drugs are illegal, a drug-free festival, however, is an utopia. For law enforcement it is necessary to rapidly determine whether controlled substances are involved. This is a very challenging task since drug of abuse appear in different physical forms such as tablets, powders, crystals, pastes, etc., and are not always easy to distinguish from medication. The aim of this study was to compare the performance of two devices using spectroscopic techniques, Raman and Fourier transformation-Infrared (FT-IR) for the on-site identification of drug seizures.

Methods: Drugs samples were measured through the package with a handheld Raman instrument (Rigaku Progeny). Thereafter, the homogenised samples were analysed with a portable FT-IR spectrometer (Alpha II Brüker). In the case of MDMA-tablets, a chemometric model was applied on the FT-IR spectra for the estimation of the dose. The on-site test results were confirmed in the forensic laboratory with reference techniques: gas chromatography (GC) combined to mass-spectrometry (MS) or a flame-ionization detector (FID). In total 166 samples of which 90 tablets, 53 powders, 16 crystals and 7 liquids were analysed.

Results & Discussion: MDMA (59%), cocaine (11%) and ketamine (9%) were the top 3 drugs seized. The majority of XTC-like tablets contained MDMA but also fake tablets (without active compound) and tablets with another active drug (such as ketamine and 2C-B) were detected. The Raman technique was suitable for powders and crystals (sensitivity of 100 and 81% respectively). In comparison to FT-IR, Raman performance was lower for the analysis of liquids (sensitivity of 71%) and 'ecstasy'-like tablets (sensitivity of 41%, mainly a problem for the dark or metallic coloured tablets). Overall, sensitivities above 95% were obtained with FT-IR. The MDMA doses of the tablets,

determined on-site with the FT-IR combined with a chemometric model, ranged between 52 and 336 mg MDMA hydrochloride (median dose of 172 mg). These quantitative results were in agreement with the GC-FID results (dose range of 77 to 266 mg; median 169 mg). Taking into account an on-site warning limit of 200 mg MDMA-HCl, only 3 tablets were incorrectly categorized (2 overestimated and 1 underestimated) with the on-site system.

Conclusion: For a quick identification of a variety of drugs on-site, the combination of the two spectroscopic techniques Raman and FT-IR is recommended. A particular advantage of Raman is the fact that it can measure samples as such (and through transparent packaging) while for FT-IR, the powders/crystals and tablets needed to be crushed which is time-consuming and potentially dangerous. For the analysis of ecstasy-like tablets, however, FT-IR is recommended since the Raman technique was not providing an identification in almost half of the MDMA-tablets, mainly for the dark and/or metallic coloured tablets. Since performing both Raman and FT-IR was time-consuming it can be recommended for future on-site measurements to use Raman for powders, crystals and liquids. For the tablets FT-IR is the preferred method since this technique had the highest sensitivity and an estimation of the MDMA dose can be performed.

It should be emphasized that optimized detector settings, as well as updated and correct in-house libraries are essential to obtain correct results. Moreover, adequate training of the operators is necessary. Both concerning drug recognition -certainly in the ever changing drug market- and working with the spectroscopic instruments.

Ketamine – a new (or old) kid on the block: A comprehensive three-year spatio-temporal study in Belgium through wastewater-based epidemiology

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Background & Aims: Initially intended for medical purposes as anaesthetic, ketamine has become increasingly popular as a recreational drug, leading to concerns about its abuse and associated health risks. Even though there has been a marked rise in intoxication events and adverse effects since 2018, obtaining a comprehensive picture on ketamine prevalence at a population scale remains difficult due to the limited availability of data collection methods and associated challenges. For example, surveys could be biased due to non-response and fear of stigmatization. Additionally, ketamine is not mandated to be measured by law enforcement (e.g. drugs in traffic) in most countries, contributing to a lack of information on its consumption pattern. To address this gap, wastewater-based epidemiology (WBE) has been employed to estimate ketamine consumption by analysing human ketamine biomarkers in influent wastewater (IWW). The objective of this study is to assess which biomarkers are best suited for consumption measurement and to evaluate spatio-temporal trends in consumption patterns of ketamine within the northern region of Belgium, Flanders.

Methods: An analytical workflow based on solid-phase extraction and liquid-chromatography coupled to tandem mass spectrometry was developed and validated for the measurement of ketamine, norketamine and dehydroketamine in IWW. Lower limits of quantification were set at 20, 4 and 8 ng/L, respectively. Spatial comparison involved 26 locations across Flanders, including urban areas as well as more rural regions. For this purpose, composite 24-hour IWW samples collected on Monday and Wednesday between January and July 2023 (n=721) were analysed. Additionally, a temporal dataset consisting of daily IWW samples from Leuven (n=393) and Brussels (n=238) in 2021 and 2022 was examined. A time series based statistical framework was used to graphically assess temporal differences, including an autocorrelation function and Seasonal and Trend decomposition using Loess decomposition. Considering the limited use of ketamine as an anaesthetic within hospital environments in Belgium and the implementation of wastewater filtration systems in medical facilities, the proportion of licit ketamine consumption can be regarded to be minimal. All concentration measurements were transformed in population-normalized mass loads (PNML), by multiplying the concentration in IWW with the flow rate and subsequently dividing by the population size of the catchment area. The PNML serve as proxy for human consumption of a specific population within a particular area and time frame, expressed as mg/day/1000 inhabitants. For temporal comparisons, dynamic population equivalents based on mobile phone data were used for refining back-estimations.

Results & Discussion: Ketamine and norketamine were identified as preferred biomarkers due to their high detection frequencies (98% and 96% of all examined samples, respectively), while dehydronorketamine was excluded from temporal analysis owing to lower detection frequency (76%). Overall, the ketamine/norketamine ratio showed a stable temporal pattern in both locations, highlighting a similar occurrence of both biomarkers in spatio-temporal trend evaluation. On specific timepoints, sudden increases were observed in the ketamine/norketamine ratio,

potentially suggesting direct disposal of ketamine in the sewer system, further demonstrating the need for appropriate human metabolic biomarkers. Spatial analysis revealed median PNMLs ranging from 8-105 (ketamine), 4-47 (norketamine), and 1-15 (dehydronorketamine) mg/day/1000 inhabitants with substantial consumption in both urban and rural areas. Higher consumption was observed in the largest cities of each province, while regional differences were observed with increased consumption in the east of Flanders. Temporal analysis showed no general or seasonal trends in both examined locations, but a significant increase (58%) in consumption during weekends was noted. When comparing with historical IWW data available in three analysed locations, ketamine use increased 7-fold, 12-fold and 15-fold in 2023 compared to 2012 in the catchment areas Brussels-North, Antwerp-South and Deurne, respectively.

Conclusion: For the first time in Belgium, the potential of WBE as a complementary surveillance method to monitor spatio-temporal trends in ketamine consumption is demonstrated. It can be concluded ketamine is currently widely consumed throughout Flanders as evidenced by a substantial quantification frequency and significant PNML levels, encompassing both urban and rural areas, with higher consumption rates observed in urban centres, particularly province capitals. There were no discernible seasonal or longitudinal trends, indicating a stable, normalized, year-round consumption. A distinct increase in consumption during weekends suggests a potential association with recreational activities. In future WBE studies, it is recommended to measure norketamine as a an appropriate biomarker for ketamine consumption as norketamine can be more consistently associated with human consumption compared to measuring the parent compound itself.

AM O-3

Insights from four years of wastewater surveillance for novel psychoactive substances from up to 20 countries

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Background & Aims: Novel Psychoactive Substances (NPS) are a complex addition to the international drug market. Their initial designation as 'legal highs' intimated safe and licit consumption in place of conventional illicit drugs such as methamphetamine, ecstasy, cocaine and cannabis. Legislation has been introduced to curb their use due unknown effects and safe dose sizes. Nevertheless, more than 1200 substances have been reported to the United Nations Office of Drugs and Crime Early Warning Advisory on NPS from all continents. With new NPS being identified every year, it is crucial from a public health standpoint to continue to monitor these compounds. Wastewater analysis has emerged as a complementary tool to provide community level consumption information. The aim of this work was i) to understand international spatial and temporal trends in the use of NPS through the sampling of wastewater every New Year period (i.e end of December – beginning January) from up to 20 countries over a four-year period; ii) to examine if preferences for NPS drugs change from year to year and iii) to establish whether data on NPS obtained from wastewater analysis is comparable to other data sources.

Methods: Influent wastewater was collected in >50 sites from 20 countries including Australia, New Zealand, China, Republic of Korea, United States, Canada, Nigeria, Brazil, Spain, Iceland, Italy, Greece, Slovenia, Cyprus, Sweden, France, Belgium, Germany, United Kingdom and Czechia. Samples were collected over a one week period during the New Year period between 2019/20 and 2022/23. These sites included small towns, large cities as well as places

with a known influx of holiday makers. Samples (100 mL) were loaded onto solid-phase extraction cartridges in the country of origin and sent to Australia for elution and analysis. All samples were analysed using a targeted triple quadrupole liquid chromatography - mass spectrometry (LC-MS/MS) method. Concentrations were calculated using the isotope dilution method, and an 8-point calibration curve with concentrations ranging from 0.5 – 500 ng/L. As labelled internal standards were not available for the NPS in this work, a surrogate internal standard was utilised. To estimate excreted mass loads, flow rates and population were provided from each wastewater treatment plant catchment area. The method was updated annually, based on the literature and discussions with national and international forensic and health agencies.

Results & Discussion: A total of 27 NPS were found across the four years of this project, from a range of classes including synthetic cathinones (e.g. eutylone, N,N-dimethylpentylone, 3-methylmethcathinone), designer benzodiazepines (e.g. bromazolam, etizolam, clonazolam), synthetic opioids (e.g. protonitazene), phencyclidine-type substances (e.g. 2F-deschloroketamine, 2-oxo-PCE) and plant-based compounds (e.g. mitragynine). Across the four years, specific temporal and spatial trends could be ascertained. For example, 3-methylmethcathinone was primarily found in sites in Europe, with the 2020-21 period having the lowest mass loads – coinciding with the strictest COVID-19 restrictions – before rebounding in the 2021-22 period. This was in agreement with data collected by the European Monitoring Centre for Drugs and Drug Addiction. Eutylone was found to be consistently high in sites from New Zealand from 2020-21 to 2022-23, with the COVID-19 pandemic having limited effect, similar to findings from the New Zealand drug checking program, Know Your Stuff. Mitragynine was found in highest levels in sites from the United States, but also found in most other sites, with increases in mass loads seen between 2021-22 and 2022-23. The impact of legislative measures on NPS was also seen, with significant decreases in 3-methylmethcathinone found in the European sites in 2022-23, after it was controlled. Similarly, 2F-deschloroketamine had a reduction in mass loads in a site in China, after it was controlled.

Conclusion: Monitoring and surveillance of NPS internationally is an ongoing and complex problem. This wastewater-based study provides an insight into the NPS market internationally over the past four years, including before, during and after the COVID-19 pandemic. By collecting samples at the same time each year, a 'snapshot' of NPS use is observed, revealing the dynamic nature of the NPS market. These data indicate the promise of more systematic wastewater analyses to identify and monitor trends in the use of specific NPS in different populations and identify temporal and spatial patterns in their global spread of use.

Comparison of four experimental setups for bias and precision studies in method validation according to different guidelines exemplified for a LC-MS/MS assay for morphine, hydromorphone and their metabolites in human plasma

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Background & Aims: Bias and precision are key parameters for method validation of quantitative analytical methods. Method validation guidelines relevant in forensic toxicology and related fields, agree that bias and precision studies should employ experimental setups with replicate analysis of QC samples over several days. However, there is no general agreement on the minimum numbers of days and replicates per day to be included. Therefore, the aim of the presented work was to systematically compare bias and precision results for a LC-MS/MS method for determination of morphine and hydromorphone as well as their major metabolites in plasma obtained with the experimental setups suggested by the validation guidelines of the Society of Toxicological and Forensic Chemistry (GTFCh; 2 replicates on each of 8 days), the Standard 036 issued by the American National Standards Institute/AAFS Standards Board (ANSI/ASB; 3 replicates on each of 5 days), and the recommendations for bioanalytical method validation of the US Food and Drug Administration (FDA; 5 replicates on each of 3 days).

Methods: The assay covering morphine, hydromorphone, morphine-3- and -6-glucuronides, normorphine, norhydromorphone, hydromorphone-3-glucuronide and dihydromorphine, employed protein precipitation of serum samples followed by UPHLC- MS/MS analysis on a triple quadrupole instrument operated in MRM mode. Bias and precision experiments included analysis of QC samples at four concentration levels across the respective calibration ranges. For each QC sample, 6 replicates (a-f) were analyzed on each of 10 days (D1-D10). Based on the results from these analyses, repeatability (within-day precision) and time-different intermediate precision (laboratory precision) were calculated as %RSDs and bias as percent deviation of the mean from the respective nominal value as detailed in the GTFCh and ANSI/ASB guidelines. Three different sets of values were calculated for the GTFCh (D1-D8, ab; D1-D8, cd; D1-D8, ef) and FDA (D1-D3, a-e; D4-D6, a-e; D7-D10; a-e) designs and four for the ANSI/ASB design (D1-D5, a-c; D1-D5, d-f; D6-D10, a-c; D6-D10, d-f). Additionally, results were calculated for the entire dataset (D1-D10, a-f). The difference between maximum and minimum estimates for repeatability, intermediate precision and bias within the subsets of each of the three experimental setups (GTFCh, ANSI/ASB, FDA) were calculated and compared between setups. Smaller differences (i.e., narrower ranges) of results within the same setup were considered indicative of more consistent estimates of the respective validation parameters.

Results & Discussion: For the entire dataset (D1-D10, a-f), bias results ranged from -20.1% to 15.6%, repeatability results from 3.0% to 8.9%, and intermediate precision from 3.5% to 11.8%. For the 10 different subsets (3 GTFCh, 4 ANSI/ASB, 3 FDA) bias results ranged from -22.9% to 17.0%, repeatability from 2.0% to 12.9%) and intermediate precision from 2.2% to 15.9%. For the 32 differences (eight analytes x four concentration levels) for each of the validation parameters between the lowest and highest estimates obtained within each of the experimental setups results were as follows: For bias, the lowest differences were observed 16 times with the GTFCh setup, 11 times with the FDA setup, and 5 times with the ANSI/ASB setup. For repeatability, the lowest differences were observed 13 times with the FDA setup, 12 times with the GTFCh setup and 7 times with the ANSI/ASB setup. For intermediate precision, the lowest differences were observed 22 times with the GTFCh setup, 8 times with the FDA setup and 2 times with the ANSI/ASB setup. The finding that smaller differences for intermediate precision were most frequently observed using the GTFCh setup is in line with theoretical expectations, since this design is associated with highest number of degrees of freedom (df) for the factor "day" (df=7 for GTFCh vs df=4 for ANSI/ASB and df=2 for the FDA design), which generally has the largest contribution to this parameter.

For the entire dataset, 2 estimates for bias slightly exceeded the acceptance limits (±20% at all concentrations for ANSI/ASB; ±20% near LLOQ and ±15% at other concentrations for GTFCh and FDA). All other estimates for bias, repeatability and intermediate precision were within these limits. Estimates for the subsets exceeded the respective acceptance limits for bias 4/96 times (4.2%; GTFCh and FDA) and 7/128 times (5.5%) for the ANSI/ASB setup. For repeatability and intermediate precision, all estimates fulfilled the respective acceptance criteria with exception of one intermediate precision estimate in the FDA setup.

Conclusion: In the studied datasets, the GTFCh experimental setup covering more days with fewer replicates provided somewhat more consistent bias, repeatability, and intermediate precision estimates compared to the ANSI/ASB and FDA setups covering fewer days with more replicates, but a similar total number measurements (n=16 for GTFCh vs. n=15 for ANSI/ASB and FDA). However, all three approaches yielded essentially identical findings regarding compliance or non-compliance of the mentioned validation parameters with the respective acceptance limits. Whether these observations can be genarilized is a matter of further studies.

AM O-5

Mathematics-based best practices for the method of standard addition

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Background & Aims: The method of standard addition (MSA) is an analytical strategy best used for new or seldom-encountered analytes (e.g., new psychoactive substances) and rare or complex matrices (e.g., tissues). Its interest lies in the inherent correction of matrix effects and the reduction of required validation experiments. Unfortunately, guidance on its application in forensic toxicology is scarce. In references dealing with MSA, such as the ANSI/ASB Standard 054 and published literature, calculations are not covered beyond the simple x-intercept formula. This work aims to establish mathematics-based best practices for MSA, in particular as it pertains to calibration model selection and measurement uncertainty (MU) estimation.

Methods: Experimental response curves (signal to concentration, n=283) of multiple analytes on different LC-MS/MS instrumental platforms were used to generate simulated MSA datasets, using a precision equivalent to 10% of the signal. Monte Carlo simulations were performed using R version 3.6.1 or above and RStudio® version 1.2.5001 or above. The simulated datasets were then subjected to different methods for regression model selection and MU estimation. To select the best approach, precision and accuracy of the results were evaluated, together with applicability to forensic toxicology settings.

Results & Discussion: In typical external calibration analytical methods, proper selection of the calibration model (weight, linear/quadratic) will yield the best accuracy. This is also true with MSA, despite the fact that models other than linear, unweighted are almost never used in the MSA literature.

While ANSI/ASB 054 suggested calibrators typically do not require weighting, these levels are in fact not optimal for accuracy in MSA. All weight selection techniques rely on calibrator replicates. Interestingly, in MSA, increasing the number of replicates is a better path to increased accuracy than increasing the number of calibrator levels. Replicate measurements are thus highly recommended due to the dual benefits of accuracy and weight selection. With three replicates or more, the mathematically accurate weight pattern can be used: equal to the inverse of the replicates' signal variance. If measurements are not replicated, an arbitrary choice of weight must be made. When two replicates or fewer are available, it is suggested to set the weight as the inverse of the squared signal due to the high prevalence of this heteroscedasticity pattern in analytical chemistry methods.

While conventional wisdom holds that MSA regressions can only be linear and not quadratic, a few authors have recently been challenging this claim. In a quadratic model, the concentration in the unknown sample is found by solving for the negative parabola root. Monte Carlo simulations performed show that if the dataset used is quadratic – which is often the case with LC-MS measurements – then a quadratic regression will yield more consistently precise and accurate results. However, concentration is more likely to be underestimated unless the number of replicates is increased to three or more.

An MU seldom, if ever, accompanies the MSA result in the literature. Yet, as with all other measurements, the analyte concentration does suffer from uncertainty of measurement. While several different approaches might be valid to estimate a concentration's MU in MSA, the standard prediction interval formula and coordinate swapping were tested in this work. These two methods are self-contained, meaning they only rely on the calibration results acquired for the MSA analysis, and not on extraneous measurements such as the instrument's precision in other methods. Simulations show that both calculations yield statistically similar results. In the end, the coordinate-swapping approach was favored for its ease of use and lessened theoretical concerns.

These MSA best practices were implemented in user-friendly platforms to perform calculations based on experimental data. The first platform is an Excel spreadsheet with VBA programming; the second platform is an online application coded with the R programming language (https://toxbrigitte.shinyapps.io/EZMSA/). Both platforms allow the user to visualize data, calculate the analyte's concentration in an unknown sample and its associated MU, and generate reports in PDF and DOCX formats. The online application further allows data importation and customizable report generation. Both platforms will also automatically choose the most accurate model weight and order depending on the number of replicates; furthermore, the R-based application allows the selections to be overridden if the user wishes to do so.

Conclusion: Using the mathematical theory underlying least squares regression and Monte Carlo simulations based on experimental signal-concentration response curves, some best practices for MSA use in a forensic toxicology setting were obtained. First, the regression model, including weight and order (linear/quadratic) must be selected for the data at hand. Second, accuracy and selection of the regression weight will be improved by increasing the number of replicates, rather than increasing the number of calibration levels. Finally, MU is most easily calculated using a coordinate-swapping approach.

Using these best practices together with user-friendly Excel spreadsheet or online application, toxicologists now have all the required tools to obtain accurate results from their standard addition analyses.

Development of a sensitive SPE-LC-MS/MS method for analysis of insulin variants, C-peptide and hemoglobin in postmortem vitreous humor and cerebrospinal fluid

AM O-6

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Background & Aims: Postmortem insulin analysis remains challenging due to rapid and unpredictable degradation in hemolyzed blood samples and low presence in alternative matrices. In addition, immunochemical assays suffer from high degrees of cross-reactivity and lack specificity, complicating the differentiation between endogenous insulin and exogenous uptake of analogues. Therefore, a sensitive SPE-LC-MS/MS method for analyzing eleven insulin variants, C-peptide and hemoglobin in vitreous humor and cerebrospinal fluid was developed. The included insulins were human, lispro, aspart, glulisine, glargine, glargine metabolites IM (21^{A}-Gly-31^{B}-Arg-human insulin) and M1 (21^{A}-Gly-human insulin), degludec, detemir, bovine and porcine. C-peptide is cleaved from proinsulin during the biosynthesis of endogenous insulin and is not present in pharmaceutical insulin preparations. Depending on the extraction method, hemoglobin can be used to monitor sample integrity and detect hemolysis. The aim was to overcome characteristic problems associated with the LC-MS analysis of insulins regarding solubility, carryover, nonspecific binding, ionization and fragmentation efficiency, so that the method would be sensitive enough to detect insulin concentrations in the picogram per milliliter range that have been reported for vitreous humor and cerebrospinal fluid regardless of insulin overdose.

Methods: For sample preparation and storage, protein low binding well plates and tubes were used. Glargine metabolites IM and M1 were produced from glargine by enzymatic cleavage using carboxypeptidase B. Spiked porcine vitreous humor served as the initial matrix for method development. Processing of the vitreous started with centrifugation, followed by removal of the supernatant and protein precipitation with an equal amount of methanol/acetonitrile (1:1), so that the insulin and the C-peptide were not co-precipitated. The mixture was then centrifuged again, the supernatant was removed and mixed with diluted NH_4OH. Next, a solid-phase micro-extraction protocol was developed for the Biotage Extrahera pipetting robot using Oasis MAX µElution 96 well plates containing a mixed-

mode anion exchange sorbent. The protocol started with two conditioning steps using methanol and water, followed by two loading steps of the sample. The wells were washed with diluted NH_4OH and aqueous methanol/acetic acid. For elution, a mixture of water, methanol and acetic acid was used, followed by addition of water to the eluate. The LC-MS system used subsequently consisted of an Agilent 1290 Infinity II LC coupled with an Agilent 6495 triple quadrupole MS. The chromatography was carried out at 80 °C with a flow rate of 0.3 mL/min. MassHunter Qualitative and Quantitative as well as Modde software were used for data analysis.

Results & Discussion: The SPE conditions were chosen to ensure insulin solubility and minimize non-specific binding. The use of a micro elution SPE eliminated the need for final evaporation and reconstitution of the eluate, which avoided adsorptive losses of protein. Acetonitrile and a 10 mM ammonium formate buffer at pH 2.60 were chosen as the mobile phase due to significantly lower ion suppression than TFA-based mobile phases. Various columns were tested, including two superficially porous C18 columns with pore sizes of 120 Å and 160 Å respectively, a fully porous C18 column with 300 Å and a C18-Ether column with 180 Å. Chromatographic separation of the structurally similar insulins, C-peptide and hemoglobin subunits was achieved with a Supelco BIOshell A160 Peptide C18 column (2 µm, 2.1 x 150 mm) in a run time of 10 min. This superficially porous particle packed column enabled short diffusion paths for biomolecules and offered an increased pore size of 160 Å, which, in contrast to 120 Å, was sufficient for the analysis of insulins and hemoglobin subunits. In order to achieve the best possible separation performance and peak areas, the column was primed with the supernatant of precipitated rat plasma, which resulted in an increase in the peak areas by a factor of 15 to 45. Various column temperatures and flow rates were also examined and it was found that elevated temperatures and low flow rates are beneficial for chromatographic efficiency. The chromatographic separation of the insulins avoided problems with overlapping isotope patterns. For the selection of precursor ions, the ESI source was optimized using a design of experiment approach. The seven factors of the central composite design were the drying gas temperature and flow, sheath gas temperature and flow, nebulizer pressure, capillary voltage and nozzle voltage. When selecting the mass transitions, it was important to carefully weigh the specificity of higher m/z fragments against the intensity of the lower m/z fragments in order to obtain the highest sensitivity in the matrix. Human insulin and insulin lispro, which differ only by two interchanged amino acids, could be distinguished both chromatographically and by unambiguous fragments. The detection limits for the insulins and C-peptide were between 10 and 100 pg/mL.

Conclusion: This MS based method for insulin analysis in postmortem alternative tissues overcomes the drawbacks of commonly used immunoassays while being sensitive enough to detect 'physiological' postmortem concentrations in samples not associated with overdose.

AM O-7

Wastewater-based epidemiology for measuring illicit drug use: increased evidence for early-warning type of information and correlation between different indicators for drug use

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Background & Aims: Wastewater-based epidemiology (WBE) has become rapidly evolving discipline for monitoring nearly real-time data on spatial and temporal trends at population-level illicit drug use. Despite numerous scientific papers, quite limited evidence has still been presented how population-level WBE data correlates to forensic and forensic toxicological indicators, and how wastewater can be constantly used as an early-warning tool for following national or regional drug situation. Objective was to study 1) how nation-wide WBE findings compared to number of drivers under influence of drugs (DUID), national drug seizure statistics and average drug purity information during long period of time, and 2) how long-term and recent changes in drug situation may be detected in wastewater and give early-warning type of information.

Methods: Wastewater analysis: Nation-wide sampling campaigns up to 28 wastewater treatment plants (WWTP) covering approximately 60% of all Finnish population (3.2 million out of 5.5 million) during 2012-2024 were performed. 24-h wastewater influent composite samples (n=232) were collected during 04/2013-03/2024 from Helsinki WWTP serving approximately 860,000 inhabitants. All latter samples were representative samples from Sunday to Monday morning and analysed by a validated UHPLC-MS/MS methodology. DUID: Prevalence of different drugs was studied in all suspected DUID cases in Finland (from 2012 to 2023). All cases (whole blood) were analysed using systematic protocol with validated and ISO 17025 accredited chromatographic mass spectrometric techniques. Therefore, representative national information was obtained on different drugs for suspected drivers. Drug confiscations and purity information: Seizure data measured in kilograms and drug purity information of different drugs in Finland from 2012 to 2023 were provided by the Finnish law enforcement authorities. The correlations between drug loads of amphetamine, methamphetamine and cocaine in wastewater and the corresponding DUID cases, drug seizures and purity information were calculated using Spearman's rank order correlation. All these substances are highly relevant in the context of Finnish drug scene and representative nation-wide data is available for all mentioned indicators since 2012.

Results & Discussion: Significant correlation between wastewater drug loads and DUID cases in a period of 2012-2022 was obtained for cocaine, amphetamine and methamphetamine in Finland. Correlation was higher for all studied drugs between wastewater and number of DUID cases, than for changes in drug purity or drug seizures, even though there was positive correlation between other indicators, as well. Mean cocaine mass loads in Helsinki wastewater were 18 mg/1000 persons/day in 2013, 47 in 2016, 116 in 2019, 218 in 2022 and 330 in 2023 showing constant increase of cocaine use in the capital area of Finland. The number of cocaine-related DUID cases during the same period increased from 81 (2013) to 963 (2023) in Finland. Law enforcement drug seizures of cocaine increased under the same period from 4.5 kg (2013) to 106 kg (2023), and average purity from 43% (2013) to 75% (2023). Even though cocaine use is still highly concentrated in the capital area of Finland, the use is also spreading to other parts of the country according to WBE.

For new psychoactive substances (NPS), synthetic cathinones have been the most popular NPS among problematic drugs users in Finland during the last decade. A few synthetic cathinones were routinely analysed in all cases (samples) in both WBE and DUID. A selection of relevant cathinones was based, e.g., qualitative monitoring of wastewater and suspected drivers, findings from other drug testing and toxicology, seizure data, as well as information obtained from user forums. For alpha-PVP and MDPV, systematically analysed quantitative data is already available since 2013, while, e.g., alpha-PHP/PiHP were later added on routine monitoring. Alpha-PVP mass loads in Helsinki wastewater were 4.4 mg/1000 persons/day in 2013, 1.3 in 2016, only 0.7 in 2019, but bounced back 4.0 in 2022 and were at a record-high level of 12 in 2023. The number of alpha-PVP positive DUID were 210 in 2013, decreased to 95 in 2019 and, similarly to wastewater, were at a record high level of 641 cases in Finland in 2023. Similarly to WBE and DUID findings, drug seizures for alpha-PVP were at a record-high level of 9 kg in Finland in 2023. Since 2018, an average purity of alpha-PVP has been over 90% each year. For MDPV, use (wastewater, DUID) or supply (seizure data) has not been recorded since 2016. According to nation-wide wastewater monitoring campaigns, use of synthetic cathinones had much more spatial and temporal variation than traditional illicit drugs, such as amphetamine or MDMA.

Conclusion: Multiple times during the last 12 years WBE has provided early-warning type of information on recent trends in drug use. These phenomena have later been confirmed by other indicators. Recent WBE data shows record-high alpha-PVP and cocaine use in the capital area of Finland. The number of DUID cases of amphetamine, methamphetamine and cocaine showed significant correlation to population-level drug use measured by WBE. Correlation was much higher than for other commonly used indicators, such as law enforcement drug seizure statistics.

Scientific Session 11 – Postmortem toxicology

08:30 – 10:00 Friday, 6th September, 2024

Chair: Dimitri Gerostamoulos, Lana Brockbals

PM O-1

Ethanol production in the gut: An autopsy case

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Background & Aims: An 82-year-old female was taken to her daily senior day care facility. Nurse at the day care facility attempted to take her vitals but noticed she was not breathing and called for an ambulance. Upon arrival at the hospital, she was found to be severely dehydrated and her blood tests came back extremely poor, and she was pronounced dead soon after. Her body was subjected to autopsy for suspicious death, and upon autopsy it was obvious she had not been taken good care of. Here we present a peculiar case where postmortem toxicology results detected ethanol only in the stomach content, and our aim was to investigate the cause of ethanol production.

Methods: Alcohol analysis: Headspace (HS) alcohol analysis was performed on an HP6890 gas chromatograph system with flame-ionization detection (GC-FID) (Hewlett-Packard, Palo Alto,CA, USA). The analytical column used was an SH-Wax fused-silica capillary column (30 m x 0.32-mm i.d., 0.25-µm film thickness) (Shimadzu, Kyoto, Japan). Helium was used as the carrier gas. One-hundred microliters of specimen sample (right heart blood or stomach content) was mixed with 5 mg/mL sodium dithionite, 0.05% 1-butanol (internal standard), and distilled water. After heating at 80°C for 10 minutes, 0.5 mL headspace was injected for HS-GC-FID analysis.

Microbiology: Specimen samples were inoculated into Pearlcore Potato Dextrose Agar (Eiken Chemical Co., Ltd., Tokyo, Japan), Candida II Agar medium (Becton, Dickson and Company, Franklin Lakes, NJ, USA), and Pearlcore Trypto-Soy Agar (Eiken Chemical Co., Ltd.) and cultured on an aerobic condition at room temperature for five days.

For enrichment culture of fungi, samples were inoculated into Sabouraud-Dextrose Broth with Lecithin & Polysorbate 80 "DAIGO" (SHIOTANI M.S. CO., LTD, Hyogo, Japan) and cultured on an aerobic condition at room temperature for five days. For enrichment culture of bacteria, samples were inoculated into Pearlcore Tryptone Soya Broth (Eiken Chemical Co., Ltd.) and cultured on an aerobic condition at 37°C for two days. After cultivation, samples were centrifuged at 8000 *g* for five minutes and the supernatant (headspace) was subjected to alcohol analysis. Isolation of bacteria and fungus from the specimen samples, as well as pure culture isolation were obtained using the streak-plate method.

Results & Discussion: External and internal examinations revealed no apparent trauma to the brain or any internal organs. Systematic toxicological analysis by gas chromatography-tandem mass spectrometry (GC-MS/MS) on postmortem blood was negative for drugs of abuse. Histopathology revealed no gross findings indicative of bacterial or fungal infection, or associated with any other events that could have attributed to the cause of death. During autopsy a semi-sweet, alcohol-like odor was noticed from the stomach content despite absence of any record of alcohol consumption by the decedent, so stomach content was also subjected to alcohol analysis in addition to blood (no urine was available).

Alcohol analysis: HS-GC-FID analysis detected ethanol below limit of quantitation (LOQ) for blood, but the ethanol concentration in the stomach content was 230 mg/dL. Headspace samples of the same specimens were re-run on GC-MS to confirm that the detected peak was in fact ethanol. Since the decedent had been bedridden and been taken care of by her son as well as taken daily to senior daycare, alcohol consumption was unlikely. The noticeable semi-sweet odor from the abdominal area at the time of autopsy led to suspicion of bacterial or fungal infection. Therefore, specimen samples were further subjected to microbiological analyses.

Microbiology: Upon HS-GC-FID analysis of the supernatants collected from the enriched cultures, ethanol was detected only from the fungal culture and not from the bacterial culture. Isolation of the fungus was therefore attempted to determine its identity. Direct isolation culture from the specimens collected from autopsy resulted in successful isolation from the stomach content; fungus isolated from the stomach content was thus speculated to be the predominant fungus. Since ethanol was also detected from the culture supernatant of this fungus isolate, the pure culture medium was sent for yeast DNA analysis for species identification. Species identification using 16S rDNA sequencing was custom ordered to TehnoSuruga Laboratory Co., Ltd. (Shizuoka, Japan) revealed that the yeast species responsible for the ethanol production in question was *Candida glabrata*.

Conclusion: We encountered a peculiar autopsy case in which ethanol was detected at a high concentration only in the stomach content. Microbiology confirmed that this ethanol production in the stomach was caused by yeast species *Candida glabrata*, which is a first report in human postmortem specimen. The cause of death, however, was circulatory failure resulting from severe dehydration due to continued inflammation such as bedsore infections.

PM O-2

Drug-related deaths at Australian music festivals

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Background & Aims: Illicit drug use is overrepresented in music festival attendees compared with the general population. Drug use often involves a wide range of substances with the potential to cause drug toxicity. Law enforcement-centred strategies intended to deter drug use and supply at these mass gatherings have been implemented throughout Australia. However, many have been criticised for their lack of effectiveness, with evidence suggesting that they can inadvertently increase the risk of drug harm. Drug deaths are often multifactorial, providing added challenges in the development of prevention strategies. This study aimed to determine the frequency of deaths involving alcohol and other drugs at music festivals in Australia and to identify potential risk factors that may inform future harm reduction strategies.

Methods: A descriptive case series study was conducted to investigate drug-related deaths at concerts and festivals throughout Australia using the National Coronial Information System (NCIS), a national repository of cases reported to the Australian coroner. Cases were identified by structured query language keyword searches which included the names of over one hundred recent music festivals. Only cases that were no longer under investigation by the coroner and where the death occurred between 1 July 2000 to 31 December 2019 (Queensland from 1 January 2001) were included. Cases were excluded where alcohol or illicit drugs were not detected; the death occurred >24 hours post-festival attendance; or where the event was a religious or cultural festival. Data collected in each case included the circumstances of death, autopsy findings, toxicology results, medical and drug use history (where available), the festival location and duration, whether clinical intervention was received, and the medical

cause of death. Unadjusted estimates of association were computed for the characteristics and circumstances of each death. Logistic regressions were used to obtain adjusted odds rations are results were considered statistically significant if $p < 0.05$. All statistics were computed using GraphPad Prism 9. Ethics approval was granted by the Victorian Institute of Forensic Medicine (VIFM) Human Research Ethics Committee (ECO9/2019) and the Department of Justice Human Research Ethics Committee (JHREC; M0247, CF18/1281).

Results & Discussion: There were 64 deaths, of which most involved males (73.4%) aged in their mid-20s (range 15-50 years). The most common cause of death was MDMA toxicity, with hyperthermia (n=8) and excess fluid intake (n=6) both reported as contributing factors to death in these cases. Most deaths were unintentional, with less than a fifth of cases (17.2%) involving intentional self-harm. The most commonly detected drug was MDMA (65.6%), followed by alcohol (46.9%) and cannabis (17.2%). This reflects the reported rates of drug use for Australian festival attendees from survey data, except for alcohol which was detected in a lower proportion of cases than was expected. The MDMA concentration among these deaths ranged up to 3.6mg/L, exceeding the range usually associated with toxicity (median 0.5mg/L, range 0.01-3.6mg/L). Most cases involved the use of two or more drugs (including alcohol). Clinical intervention was involved in 64.1% of cases and most festivals occurred in inner city locations (59.4%). The higher proportion of deaths occurring at multiday events (56.2%) suggested that additional harm reduction strategies may be required at festivals with onsite camping.

Conclusion: This study was the first to summarise drug-related deaths occurring following attendance at Australian music festivals, using coronial records. The findings suggest that these deaths typically involve young people using multiple illicit substances in combination with alcohol. Most are unintentional and could potentially be prevented through the implementation of a range of harm reduction strategies, including mobile medical care, drug checking services, and increased consumer education and awareness.

Death of an infant due to the administration of diphenhydramine and zopiclone

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PM O-3

Background & Aims: A 22-month-old child was found lifeless in the nursery at morning by the mother underneath a fallen chest of drawers. According to the mother, the child had been lying in one of the drawers. It was initially assumed that the child had climbed onto the chest of drawers and died due to injuries from the fall. During the forensic post-mortem examination, there were no indications that the child had been alive at the time of the accident since the abrasions and marks visually appeared most likely postmortem. As no macroscopic cause of death could be determined during autopsy, further toxicological examinations were commissioned. The previous history and the interrogations of the parents did not initially reveal any relevant intake of medication or possible drug intake.

Methods: Untargeted analyses were performed in stomach content, heart blood and gallbladder fluid (GC-MS; LC-MS/MS; LC-QToF). As too little femoral vein blood and urine was available, alcohol was determined in muscle tissue as usual using HS-GC-FID, other volatile substances were screened in heart blood, lung and brain tissue using HS-GC-MS. The 24 cm hair strand of the child was analyzed for drugs and medication in segments of 2 cm (LC-MS/MS). Quantifications were performed using LC-MS/MS.

Results & Discussion: Diphenhydramine and zopiclone were detected in stomach content and heart blood. In the gallbladder fluid, only diphenhydramine was found. Quantification in femoral venous blood revealed high concentrations of 0.58 mg/l for diphenhydramine and 0.20 mg/l for zopiclone. Hair was positive for diphenhydramine, zopiclone and tramadol with varying concentrations in the nine segments of 2.5 – 5.4, 0.47 – 2.4 and 0.65 – 3.5 ng/mg, respectively, methorphan and acetaminophen were also detected. Concentrations of diphenhydramine and zopiclone were higher in proximal segments while concentrations of tramadol were lower. Mother's hair was positive for tramadol and zopiclone (>100 ng/mg and 2 ng/mg in the 2 cm proximal segment) and other drugs, father's hair for tramadol (0.25 ng/mg in 5 cm chest hair) and the hair of the 7-year old sister for diphenhydramine and zopiclone (2.8 and 0.7 ng/mg, respectively as highest concentration in a segment). From the results a history of drug administration to the deceased as well as its sister by the substance misusing mother was considered. As cause of death an acute overdose of diphenhydramine and zopiclone was assumed.

Diphenhydramine is an over-the-counter sedative antihistamine which is in children used to treat nausea. Sedation is the major side effect which is the cause of its potential abuse. Recent studies suggest that sedative antihistamines are not not as harmless as is generally assumed. Seizures or arrhythmia can occur, especially in children or at higher doses. Zopiclone is used as hypnotic in adults and may have contributed to the toxic effects of diphenhydramine.

The inconsistencies in the mother's statements, the autopsy findings and the toxicologically results subsequently led to a homicide charge against the mother. Faced with the toxicological results she admitted that she had regularly administered her zopiclone medication and later also diphenhydramine to her child, already shortly after birth, because she could not cope with her living situation and child care. Due to habituation effects in her child, she repeatedly increased the dosage of diphenhydramine and zopiclone over time. She also admitted giving diphenhydramine to her second, elder child and she confessed that she herself had been highly addictive to tramadol for years. The court was convinced that the administration of drugs caused the infant's death. However, this was assumed to be accidental and therefore the mother was not charged with homicide, but bodily harm with fatal consequence.

Conclusion: The present case illustrates that toxicological investigations are necessary to elucidate the fatal course of drug abuse in a family by an overstressed mother and it also shows the danger posed by medication considered harmless.

PM O–4

Further evaluation of bladder wash as an alternative specimen for postmortem toxicology: comparison to screening results of urine and kidney tissue

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Background & Aims: Urine specimens represent the sample of choice for screening analyses in forensic toxicology given its high concentrations and presence of drug metabolites. However, urine collection in postmortem cases is not always possible. In these circumstances, alternative specimens need to be considered for screening purposes. The Zurich Institute of Forensic Medicine (ZIFM) has used kidney tissue for this purpose, in addition to a multi-target blood analysis. However, the handling of tissue in the laboratory is more complex than the analysis of biofluids. At the 2022 TIAFT meeting, colleagues from San Francisco presented bladder wash as an interesting alternative, i.e., the collection of a saline solution used to wash the bladder wall, collecting residual urine and/or dried contents of the bladder wall. Still, knowledge about routine applicability – particularly in general unknown screening approaches – is poor for that specimen. We, therefore, aimed to systematically compare results of immunoassay analysis and an untargeted LC-MS/MS screening approach between urine, kidney tissue, and bladder wash as a new alternative in a paired manner.

Methods: The study included 60 consecutive postmortem cases. If available, urine, bladder wash, and kidney tissue were collected from each case. Urine was collected in polypropylene tubes as usual. Bladder wash was obtained after complete emptying of the bladder, followed by flushing 10 mL of a 0.9% NaCl solution along the bladder wall. A piece of kidney tissue (ca. 50 g) was preserved in a plastic tub. The final study collective consisted of n=34 complete cases of paired urine, bladder wash, and kidney tissue (subset 1), n=13 cases of paired urine and bladder wash (subset 2), and n=13 cases of paired bladder wash and kidney specimens (subset 3), respectively. Kidney tissue (ca. 5 g, grinded) was placed into a tube of dialysis membrane and left for 24 h for dialysis against water (40-80 ml; replaced twice after 3 h prior to overnight dialysis). Dialysate solution was evaporated and reconstituted to a final concentration of 1 g tissue/mL. All samples were analyzed by immunoassay (CEDIA; Indiko Plus, Thermo Fisher) targeting amphetamines, benzodiazepines, cannabis, cocaine, methadone, and opiates. In addition, specimens were diluted and filtered (200 µl specimen + 200 µl mobile phase, aqueous/organic 95:5, v/v; filter vials Mini-UniPrep™ PTFE Filter (0.45 µm)) prior to untargeted LC-MS/MS screening covering about 1500 drugs and their metabolites (Bruker Toxtyper; reversed phase chromatography, ion trap mass spectrometer; ESI+; data-dependent acquisition; automated library search Maurer/Wissenbach/Weber database). The sensitivity of drug detection was calculated based on the percentage of drugs detected in bladder wash compared to drugs detected in urine and kidney tissue within each subset, respectively.

Results & Discussion: Seven of the 60 initially included cases were negative for any drug (3 from subset 1, 3 from subset 2, and 1 from subset 3). Immunoassay analysis of subset 1 revealed the following percentages of positive bladder wash samples in comparison to urine and kidney tissue, respectively: amphetamines 40%, 100% (excluding two cases with amphetamine immunoassay positive in kidney tissue due to putrefaction) (n=5); benzodiazepines 69%, 90% (n=13); cannabis 44%, 0% (n=9); cocaine 100%, 100% (n=7), methadone 67%, 100% (n=5); opiates 67%, 75% (n=9). Using the untargeted LC-MS/MS screening approach, a total of 79% (compared to urine) and 103% (compared to kidney tissue) of all drugs tested positive in bladder washes in subset 1. Drugs less often detected in bladder wash compared to urine and kidney tissue were, e.g., codeine, diazepam, flurazepam, midazolam, and olanzapine. Conversely, sensitivity in bladder wash was higher compared to kidney tissue, e.g., for methylphenidate and mirtazapine. Overall, the number of metabolite spectra detectable per case was generally lower in bladder wash, but also in kidney tissue, compared to urine. For instance, only half the number of metabolite spectra were present in bladder wash (1-10) and kidney tissue (1-4) compared to urine (3-14). While neither bladder wash nor kidney tissue

obtained the same drug detection rate as urine, bladder wash generally gave comparable results to kidney tissue. Findings from subsets 2 and 3 were consistent with the results from subset 1 and did not provide any indication that recent bladder emptying during autopsy affected the results compared to voided or dehydrated bladder prior to autopsy.

Conclusion: Urine remains the gold standard for screening analysis. Sensitivity for drug detection in bladder wash using common toxicology screening approaches (immunoassay, untargeted MS/MS) is lower compared to urine specimens. The same is true for kidney tissue. Still, comparable results can be obtained with bladder wash as with kidney tissue. Given the easier handling of bladder wash in the laboratory (basically identical to urine specimens), its use proved to be superior to kidney tissue and represents a promising new strategy for cases without urine samples. Further evaluation of more sophisticated sample preparation (re-concentration) of bladder washes is necessary and might further increase sensitivity for this specimen.

Forensic Entomotoxicology: Assessing the potential of necrophagous larvae for post-mortem toxicological analysis, using diazepam in a minipig model

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Background & Aims: Entomotoxicology draws upon the fields of forensic toxicology and entomology. One of its primary focuses is the use of insect specimens as an alternative matrix for post-mortem toxicological analysis of decomposed bodies to obtain information on the past drug use of the deceased. However, the successful identification of drugs in necrophagous insects may depend on pharmacokinetic processes in the larvae that feed on the body. To test this hypothesis, the authors established the first minipig model for entomotoxicological analyses to detect the benzodiazepine diazepam in blowfly larvae. Minipigs are a special strain of miniature domestic pigs commonly used in biomedical research due to their anatomical, physiological, and pharmacokinetic similarities to humans. Göttingen Minipigs are the most frequently used breed.

Methods: The experiments were conducted during July and August 2023. Two Göttingen Minipigs, each weighing approximately 60 kg, were orally administered 25 mg/kg of diazepam. The minipigs were euthanised with pentobarbital after one hour, which is the estimated time of maximum plasma levels of diazepam in Göttingen Minipigs according to literature sources. The first blood and liver samples were collected immediately after euthanasia on day 0. Minipig carcasses were immediately transported to an outdoor experimental site and placed in cages to facilitate post-mortem decomposition and insect colonisation. Insect traps were positioned around each cage to collect wandering larvae. Where available, porcine samples and blowfly larvae were collected on 13 occasions over 69 days, starting on day one. Cardiac and peripheral blood were obtained by blind puncture of the heart and jugular vein. Liver samples were obtained through a small abdominal incision, which was closed again after each sampling. Larvae were collected from the highest colonised areas of the minipigs, killed by immersion in hot water and stored with all other samples at -20°C until analysis. Liver samples of 1 g each were homogenised with isotonic sodium chloride (NaCl) solution, using an Ultra-Turrax®. Approximately 100 mg of larvae per sample were homogenised with metal beads in isotonic NaCl solution, using a Precellys® 24 tissue homogeniser. Liver homogenate and blood were subjected to protein precipitation with acetonitrile. Larval homogenate was extracted using Oasis® PRiME HLB cartridges under positive pressure. Extracts were reconstituted in eluent and analysed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) on an Agilent 6490 triple quadrupole LC/MS system. Quantification was performed using the ratio of the peak areas for diazepam and its metabolites to the peak areas of the corresponding deuterated standards. The limits of detection for diazepam, nordazepam, oxazepam, and temazepam are 13, 5, 11, and 6 µg/L, respectively.

Results & Discussion: Peripheral blood was available until day five, cardiac blood until day 13, and liver until day 27. Larvae, on the other hand, were available until the termination of the experiment on day 69. Diazepam and its metabolites were successfully identified in all larval samples, even several weeks after porcine samples were no longer available for toxicological analysis. There was no correlation between substance concentrations in larvae and those in porcine samples. This was anticipated, as several factors, including the developmental stage of larvae and the area from which they fed during sampling, determine the amount of substance they consume and store. Interestingly, the ratio of nordazepam and oxazepam to diazepam in larvae was significantly higher than in porcine samples, suggesting that necrophagous larvae metabolise diazepam, and possibly other drugs of forensic importance as well. This finding is relevant in forensic casework because the high concentrations of nordazepam and oxazepam in larvae may suggest that the deceased consumed these substances in addition to, or instead of, diazepam. This caution may also apply to other pharmacologically active metabolites available on the drug market, *e.g. O*-desmethyltramadol.

The concentration of diazepam was unusually high in liver samples compared to those in blood and larvae. Post-mortem redistribution of diazepam from the stomach contents into the liver tissue may have contributed to this finding, considering that the minipigs were euthanised shortly after receiving a high oral dose of diazepam. Oxazepam was only detected in the liver samples taken on day 27, whereas significant concentrations of oxazepam were found in all blood samples. This is likely due to the high rate of glucuronidation in minipigs and the absence of a hydrolysis step during sample preparation.

Conclusion: This work presents the first minipig model for assessing the suitability of necrophagous larvae as an alternative matrix for post-mortem toxicological analysis. Diazepam was successfully identified in all larval specimens even several weeks after the last available porcine samples were taken for toxicological analysis. However, as per previous research, our results indicate that blowfly larvae can further metabolise drugs present in their food source. When applying entomotoxicological methods in real forensic cases, it is important to consider the pharmacokinetics of drugs in the larvae used for toxicological analysis. This would help avoid misinterpretation of substances consumed by the deceased.

PM O–6

Drug identification through analysis of maggots recovered from skeletonized dead body – A case study

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Background & Aims: Entomological specimens are beneficial in determining drug exposure when traditional specimens are not available for analysis. Although, hair, oral fluid, sweat and meconium are commonly used alternative specimens, exhumed or putrefied corpses pose unique analytical challenge when alternative samples are not available for analysis. Entomological specimens have been traditionally used for estimating postmortem interval in medico-legal cases. However, these specimens, especially maggots from the dead body, have not gained much popularity in forensic fraternity regarding their use as toxicological specimens.

The aim of the present study is to highlight the importance of analyzing maggots as excellent specimen for identification of drugs in scenarios where other conventional toxicological specimens are not available. In this case study maggots were submitted from a putrefied corpse to confirm the drug exposure, hence opening the horizons for use as reliable specimen in forensic toxicology.

A decomposed body of an unknown middle-aged male was found in the countryside. The dead body was skeletonized and heavily infested with maggots, which were submitted to the author's laboratory for confirmation of drug exposure. Paraphernalia and cigarettes present at the crime scene indicated possible drug abuse.

Methods: A homogenized maggot sample underwent screening for the presence of drugs of abuse through immunoassay. Qualitative identification of drugs and poisons was performed using gas chromatography mass spectrometry (GC/MS) in scan mode. A modified QuEChERS method was used to prepare the sample for GC/MS screening utilizing acetonitrile as the extraction solvent. A sorbent containing a mixture of C_{18} and specialized polymers, known as enhanced matrix removal lipid, was utilized to eliminate matrix interferences. Confirmation and quantification of opiates and benzodiazepines were carried out using GC/MS in SIM mode. Benzodiazepines underwent derivatization with MTBSTFA post liquid-liquid extraction, while opiates underwent solid-phase extraction followed by derivatization with BSTFA for confirmation.

Results & Discussion: Maggots sample showed presence of diazepam, nordiazepam, oxazepam, codeine, and morphine. According to the results, the morphine-to-codeine ratio (M/C) was greater than 15 in maggots. The Stam and Gerostamoulos model, for attribution of death to heroin, was applied to this case study to determine the possible cause of death. As per the model, current results suggest a score of 8 for toxicological evidence based on M/C, while the presence of paraphernalia and cigarettes at the crime scene scored 2 as indicative of investigative evidence. The combined score for toxicological and investigative evidence was 10, which, as per the model, is strongly suggestive of heroin-related death. Moreover, the detection of diazepam and metabolites (nordiazepam and oxazepam) in maggots is also indicative of heroin consumption, as diazepam is used as a cutting agent in street heroin.

Conclusion: Maggots collected from decomposing corpses may be considered potential evidence in forensic toxicology as they may provide useful information to conclude drug exposure. This case study supports the stability and detection of not only opiates (morphine and codeine) but also benzodiazepines (diazepam and its metabolites) in maggots, establishing ante-mortem drug exposure in putrefied corpses. Considering the significance of maggots, there is sufficient room for research to collect data on drug recovery in maggots. Furthermore, this study underscores the crucial role of toxicological analysis, especially when histopathological examination is impractical for skeletonized remains.

Post mortem distribution of isotonitazene and its three metabolites in the first lethal case observed in France

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Background & Aims: Isotonitazene (IZN) is a new synthetic opioid that was first reported in Europe in 2019. It is a highly potent synthetic opioid belonging to a class of benzimidazole analogues responsible for numerous opioid toxidrome deaths, notably in the United States. We reported the analytical exploration of the first case of IZN-related death in France in March 2023 concerning a 39-year-old man with a history of heroin regular use presenting with congestion and pulmonary oedema. We performed the determination of IZN and metabolites using liquid chromatography coupled to mass spectrometry in tandem (LC-MS/MS) to document post-mortem distribution and tropism of the drug to identify the most exposed organs after consumption.

Methods: In total, 1mL of various biological samples (after grind for organs) was aliquoted and 5µl of Internal standard (Fentanyl-D5) was added to each sample. Then, alkalinization was done by addition of 1mL of Borax buffer (10 mM, pH 10.4) followed by 3 mL of extraction solvent (70:30 N-butyl chloride, ethyl acetate). After being rotated and centrifuged for 10 min at 4,600 rpm the organic layer was transferred and dried under nitrogen at 35°C. The samples were reconstituted in 200 µL of initial chromatographic conditions and transferred into vials, analysis were realized in duplicates. The lowest limit of quantitation and detection were 0.5 and 0.1 ng/mL, respectively. Besides, full toxicological screening was performed following standard protocol. The studied autopsy samples included peripheral and cardiac blood (PB and CB), urine, vitreous humor (VH), gastric content (GC), bile, spleen, liver, brain, lung, heart, kidney, muscle, subcutaneous fat (SF), and presumed injection site (IS) as a syringe with a needle was found in a garbage.

Results & Discussion: Except therapeutic concentrations of cetirizine (28.7 ng/mL), IZN was the only toxic identified in the following matrices (ng/mL or mg/g): PB (1.17), CB (1.76), brain (21.5), lung (88), heart (20), SF (8.25), gastric contents (4.38) and presumed injection site (343). IZN was detected in urine, kidney, spleen, muscle and bile (>0.1) but not in VH. The peripheral blood concentration found was comparable with those found in the literature, confirming the very high potency and lethality of IZN in the absence of other identified toxics and causes of death. Indeed, the average concentration of IZN calculated from short series of lethal cases in Mueller F. et *al*. and Krotulski A.J. et *al*., was 2±2.15 ng/mL. Post-mortem redistribution appears intense, with a ratio of peripheral to cardiac blood concentrations of 1.5, converging with the redistribution data of the studies cited. It confirms the high distribution of IZN in the brain, lungs and heart, with respective ratios relative to PB of 18/5, 75/11 and 17/3.5 (/ bibliographic data). While these ratios exceed the averages of the literature, there is considerable variability in these ratios between published cases. That argues for high lipophily of IZN, in line with good diffusion towards brain and SF, poor diffusion in urine and kidney and no diffusion in VH. Finally, with regard to the mode of IZN consumption, the forensic physician's findings and the analysis of the injection site with a high concentration of IZN suggest intravenous administration. About the metabolites, 4-OH-Nitazene was found only in bile (6.3) and detected in urine (0.2). N-desethyl-IZN was found in CB (0.1), lung (4.1), heart (0.4) GC (0.76) and urine (0.54). The last metabolite studied was 5-Amino-IZN till detected (> 0.5) in spleen, brain, lungs, GC and urine (no quantitative data stated because its very bad stability). Conversely and in accordance to available published data, IZN and metabolites were never detected in liver. This can be explained either by intense metabolism to other secondary compounds (such as the N,O-didesalkyl metabolite), or by primary pulmonary metabolism to N-desethyl-IZN. We may assess that metabolism of IZN follows three pathways (N-dealkylation, O-dealkylation and nitroreduction) with major biliary excretion as 4-OH-Nitazene.

Conclusion: To our knowledge, this is the first confirmed case of death in France involving the consumption of IZN, including post-mortem redistribution of IZN in blood, urine and major organs. Very low blood concentrations measured and strong distribution in vital organs confirmed the high dangerousness of this opioid. Its metabolic pathway appears multiple, possibly mainly extrahepatic, and needs further elucidation.

Conflict of interest with the financial support of SFTA.

Scientific Session 12 – Novel psychoactive substances II

10:30 – 12:30 Friday, 6th September, 2024

Chair: Svante Vikingsson, Volker Auwärter

NPS O–10

In vitro structure–activity relationships and forensic case series of emerging 2-benzylbenzimidazole opioids

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Background & Aims: In response to class-wide bans on fentanyl analogues, 2-benzylbenzimidazole 'nitazene' opioids have recently emerged on recreational drug markets worldwide. With potencies exceeding that of morphine and even fentanyl, nitazene use is particularly dangerous. Although numerous nitazenes have been extensively examined, only few reports have conducted a systematic comparison of the effect of different structural modifications to the 2-benzylbenzimidazole core structure on μ-opioid receptor (MOR) activity. Furthermore, the constant emergence of new analogues, combined with increased detection and intoxication rates, signals a potentially looming nitazene crisis that requires close monitoring. Here, we performed an *in vitro* assessment of the MOR activation potential of 9 previously uncharacterized nitazenes, along with their close analogues. Specifically, we focused on MOR activation by 'ring' nitazenes (i.e., *N*-pyrrolidino and *N*-piperidinyl analogues), *N*-desethyl nitazenes and 'des'-nitazenes (lacking the 5-nitro group). In addition, we bridged the pharmacology data with case data and report on the identification and toxicological analysis of etodesnitazene, *N*-desethyl etonitazene, *N*-desethyl isotonitazene, *N*-pyrrolidino metonitazene, and *N*-pyrrolidino protonitazene in 85 forensic cases from North America and the United Kingdom.

Methods: Two complementary *in vitro* MOR activation assays were employed to evaluate the MOR activation potential of a set of 25 differentially substituted 2-benzylbenzimidazole opioids (i.e., a NanoBit®-based β-arrestin 2 recruitment assay and a GloSensor® cyclic adenosine monophosphate (cAMP) assay). Forensic toxicology drug screening (LC-(Q)TOF-MS-based), followed by quantitation of etodesnitazene, *N*-desethyl isotonitazene, *N*-pyrrolidino metonitazene, and *N*-pyrrolidino protonitazene (LC-MS/MS-based, employing a standard addition approach) in authentic casework samples was conducted by the Center for Forensic Science Research and Education (CFSRE, Philadelphia, USA).

Results & Discussion: The obtained potency and efficacy values allow to further build on previously established structure-activity relationships for nitazenes. Our results show that 'ring' modifications overall yield highly active compounds, with *N*-pyrrolidino substitutions generally being more favorable for MOR activation than *N*-piperidine substitutions. Furthermore, the importance of the nitro group at the 5-position of the benzimidazole ring was demonstrated, as its removal consistently led to a 10- to 100-fold reduction in potency. The *N*-desethyl modification is overall well-tolerated in terms of MOR activation potential, and generally lowers the potency ~6- to 14-fold. Intriguingly, *N*-desethyl isotonitazene showed an opposite trend, and was somewhat more potent than isotonitazene. Consistent with previous findings for other nitazenes and in line with what could be anticipated from the *in vitro* pharmacological characterization, blood concentrations obtained for etodesnitazene, *N*-desethyl isotonitazene, *N*-pyrrolidino metonitazene, and *N*-pyrrolidino protonitazene in the evaluated forensic case series (n = 85) were generally in the low ng/mL range. Interestingly, *N*-pyrrolidino metonitazene was most often found in combination with *N*-pyrrolidino protonitazene.

Conclusion: The comparative pharmacological evaluation of a diverse panel of nitazenes carried out in this study, combined with the identification and quantification of several of these newer nitazenes in a large forensic case series, may contribute to raising awareness, as well as strengthen preparedness and harm reduction efforts.

NPS O–11

New wine in old skins: MDMB-BINACA and its detection, metabolism and pharmacology

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Background & Aims: Among New Psychoactive Substances (NPS), Synthetic Cannabinoid Receptor Agonists (SCRA) stand out as most extensive and structurally diverse group. The driving force behind this variety is the constant addition of new, previously unseen structural moieties to SCRA, and the process of combining established structures to form new SCRA. Notable examples include interchanges between indole and indazole cores, substitutions

in side chains, and alterations in linked groups such as amino acid amides, methyl esters, or ethyl esters. A recent example of this is MDMB-BINACA, the methyl ester derivative of ADMB-BINACA (also: ADB-BINACA). Here we report biomarkers for monitoring of MDMB-BINACA consumption in urine, its metabolism, and its affinity and potency at the *h*CB_1 receptor.

Methods: Urine samples underwent glucuronidase cleavage and liquid-liquid extraction with acetonitrile. For comparison and confirmation, metabolites were created *in vitro* by incubating MDMB-BINACA with pooled human liver microsomes (pHLM) in triplicates. Analysis of urine samples and pHLM extracts was performed using LC-qTOF-MS. The affinity of MDMB-BINACA at *h*CB_1 was assessed with a competitive ^{3}H-labelled radioligand binding assay, potency and efficacy were assessed with a competitive ^{35}S-GTP-γS radioligand binding assay.

Results & Discussion: In total, 33 different metabolites resulting from 8 metabolic reactions including mono- and dihydroxylation, dihydrodiol formation, dehydration, ester hydrolysis and combinations thereof were identified in urine samples and pHLMs. Notably, mono-hydroxylation and dihydrodiol metabolites emerged as the most abundant, suitable as specific biomarkers of MDMB-BINACA consumption in urine. Furthermore, MDMB-BINACA exhibited an affinity of 3.3 nM, a potency of 29.2 nM and an efficacy of 122.9% at the *h*CB_1 receptor. Between the beginning of 2023 and March of 2024, MDMB-BINACA was detected in 3.2% of analyzed urine samples and 17.7% of urine samples tested positive for SCRAs.

Conclusion: The emergence of MDMB-BINACA contributes to the already diverse landscape of prevalent SCRA. The identification of specific urine biomarkers allows for the identification of MDMB-BINACA consumption and the differentiation between the consumption of ADMB-BINACA and MDMB-BINACA. The pharmacological properties of MDMB-BINACA indicate that it acts as a full and potent agonist at the *h*CB_1 receptor. However, its potential establishment on the drug market warrants further observation.

In vitro neurotransmitter inhibition of recently detected synthetic cathinones 2-methyl-alpha-PiHP, *N*-butyl-butylone, and *N*-cyclohexyl-butylone

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Background & Aims: Synthetic cathinones are seized in increasing quantities and are considered well-established in some European drug markets. Further, toxicity presentations and drug-induced deaths involving synthetic cathinones are on the rise. Synthetic cathinones are sold as alternatives to more common stimulant drugs (e.g. amphetamines and cocaine) and generally have similar modes of action, i.e. through monoamine transport inhibition at the dopamine, serotonin, and norepinephrine re-uptake transporters (DAT, SERT, NET, respectively). Recent additions to the synthetic cathinone class are the herein investigated 2-methly-alpha-PiHP, *N*-butyl-butylone, and *N*-cyclohexyl-butylone, that all have first emerged in 2022. 2-Methyl-alpha-PiHP was first seized in Sweden in September 2022 and has since been detected multiple times throughout 2023. Furthermore, toxicological cases (living individuals and postmortem) involving 2-methyl-alpha-PiHP were detected in Sweden within the scope of the author's forensic toxicological casework. As often seen for new psychoactive substances (NPS) there is extremely little information available on their modes of action and toxicity. Therefore, we assessed the inhibitory potential of aforementioned synthetic cathinones using an *in vitro* cell model in comparison to 3,4-methylenedioxymethamphetamine (MDMA), cocaine, mephedrone (4-methylmethcathinone) and further structural analogues; *N*-butyl-pentylone and *N*-butyl-hexedrone. This work aims to deepen the understanding of the modes of actions and potencies of these recent additions to the NPS market whilst also offering some insights into structure activity relationships. Additionally, DAT/SERT inhibition ratios, a measure of abuse potential, were investigated.

Methods: Cells expressing either human DAT (CHO-K1 cells), SERT (HEK293 cells), or NET (MDCK cells) were incubated with the respective drugs following a semi-automated and miniaturized experimental protocol. Per drug, 15 concentrations were assessed in triplicate and in three individual experiments. The amount of neurotransmitter taken up by the cells is assessed using a fluorescent dye mix (i.e. strong inhibition exerted by the tested substances resulting in lack of dye within the cells) read out by a TECAN Spark plate reader. Dose response curves were calculated and mean inhibitory concentrations (IC_{50}) determined. The resulting IC_{50} values were used to calculate DAT/SERT inhibition ratios to estimate abuse liability.

Results & Discussion: *N*-Butyl-butylone (IC_{50} 29.9 nM), *N*-cyclohexyl-butylone (IC_{50} 44.0 nM) and 2-methyl-alpha-PiHP (IC_{50} 7.55 nM) resulted in strong inhibition at DAT, surpassing the inhibitory potential of cocaine (IC_{50} 136 nM). Highest inhibitory potential at DAT was observed for 2-methyl-alpha-PiHP with an IC_{50} 18 times lower

compared to cocaine. All investigated compounds were targeting DAT over NET and showed the least inhibitory potential for SERT, overall indicating dopaminergic features being more pronounced, i.e. amphetamine-like psychostimulant effects rather than MDMA-like properties. Transporter inhibition at NET ranged from 177 nM (2-metyhl-alpha-PiHP), and 2000 nM (*N*-cyclohexyl-butylone), to 3000 nM (*N*-butyl-butylone), whilst low inhibitory potentials were found for SERT with IC_{50} values of 979 nM, 8700 nM and, >10000 nM for 2-methyl-alpha-PiHP, *N*-butyl-butylone, and *N*-cyclohexyl-butylone, respectively. In comparison, *N*-butyl-pentylone and *N*-butyl-hexedrone showed similar potencies at DAT with decreased inhibitory potentials at NET and SERT. The herein described selectivity of DAT over SERT is also manifested in the DAT/SERT inhibitory ratios, whereby higher ratios are linked to an increased abuse liability. The DAT/SERT inhibition ratios ranged from 0.4 for MDMA, over 3.5 for cocaine, to 130 for 2-methyl-alpha-PiHP and 290 for both *N*-cyclohexyl-butylone and *N*-butyl-butylone. These results indicate a high abuse potential for these newly emerging synthetic cathinones. Furthermore, dopamine selectivity has been discussed to promote aggressive and psychotic behavior. The potent synthetic cathinone 2-methyl-alpha-PiHP was detected in two mixed-intoxication autopsy cases in Sweden. For one of these cases, 2-methyl-alpha-PiHP was classified to have contributed to death.

Conclusion: This work demonstrated that newly emerging synthetic cathinones are strong monoamine-reuptake inhibitors with a selectivity for DAT over SERT. The IC_{50} values of the investigated emerging synthetic cathinones were many times lower compared to cocaine. Ultimately, this indicates great potency and a high abuse liability of the herein investigated synthetic cathinones and their potential negative health impacts, classifying them as public health threats. This is an example of the evolving market for the synthetic cathinones and stresses the need to gather information about them, since they also continue to be identified in forensic toxicological casework.

NPS O–13

In vitro γ-aminobutyric acid type A receptor activity of prescription and novel benzodiazepines detected on the European illicit drugs market

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Background & Aims: Benzodiazepines are anxiolytic and hypnotic drugs that are widely prescribed globally for conditions including anxiety, insomnia and epilepsy. Benzodiazepines are positive allosteric modulators (PAMs) of the g-aminobutyric acid type A ($GABA_A$) neurotransmitter receptors at the α+/γ2− interfaces and thereby enhance GABA-induced chloride ion flux leading to neuronal hyperpolarization. Since 2007, novel chemically analogous compounds that exhibit similar clinical and pharmacological properties to benzodiazepines have emerged on the illicit drugs market, often referred to as "novel benzodiazepines". The use of novel benzodiazepines has been increasing and is recognized as a global threat to public health by the United Nations Office on Drugs and Crime.

The exact compounds available on the illicit market is continuously evolving with 36 different novel benzodiazepines notified to the EU Early Warning System (EWS) to date. There is little to no pharmacological information available on these novel benzodiazepines, making it difficult to determine their potential harm to individuals and public health and produce accurate harm reduction advice or determine their pharmaceutical potential. The overall aim of this study was to develop and use an *in vitro* cell-based $GABA_A$ receptor activity assay that measures the electrophysiology of the receptor to determine the potency and efficacy of a series of novel benzodiazepines, including the most prevalent on the illicit market and structurally diverse compounds.

Methods: $GABA_A$ ligand concentration-response experiments were performed for reference standards of different benzodiazepines using the QPatch II (Sophion Bioscience A/S, Denmark), a fully automated patch-clamp system that provides an electrophysiological study of ion channels, and a Chinese hamster ovary (CHO) cell line continuously expressing the $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptor (B'SYS GmBH, Austria). QPlate 48X plates were used, which records the currents from 10 cells per recording site. Physiological intracellular and extracellular solutions were used. GABA and benzodiazepines were dissolved to a stock concentration of 10 mM in MilliQ H_2O (aliquoted) or DMSO respectively and diluted in extracellular solution on the day of recordings. Cells were clamped to a holding potential of -90 mV throughout the recordings, and the zero-cursor subtraction for leakage was used. An experiment consisted of 10 consecutive applications of liquids to one recording site and each compound was tested in 4-8 experiments. There were three applications of a GABA-reference (2 µM solution of GABA), six applications of benzodiazepines with increasing concentrations (1.5 pM to 4.8 nM or 0.96 nM to 3 µM, (1:5 dilutions)), then one application of GABA-reference. Cells were pre-incubated with benzodiazepines for approximately 180s before re-application alongside 2 µM GABA. All solutions contained 0.3% DMSO. The peak current in presence of GABA (averaged during 5 ms) was used for analysis, which were normalized based on the average of the experiment-specific GABA-reference (used as

baseline) and a time-matched negative control (i.e., only DMSO) to compensate for potential non-compound dependent effects in the recordings. Data was analyzed using Sophion Analyzer 7.0 and GraphPad Prism 10.

Results & Discussion: When looking at the latest novel benzodiazepines to be notified to the EU EWS, 4'-chloro deschloroalprazolam, which was notified in 2023, was actually found to be a benzodiazepine antagonist and fluetizolam, notified in 2022, was a weak PAM (EC_{50} = 149.70 nM). Bromazolam, the most prevalent novel benzodiazepine on the illicit market now, was found to have similar potency to etizolam (EC_{50} = 15.15 and 15.48 nM, respectively), which was about three times stronger than diazepam (EC_{50} = 44.3 nM). The other prevalent benzodiazepines are more potent, including desalkylgidazepam (11.43 nM), flualprazolam (4.78 nM), flubromazepam (3.04 nM), and flubrotizolam (3.01 nM).

Structure-activity relationships were also considered based on the activity of at least 38 different benzodiazepines. Despite having different base structures, all of the most potent benzodiazepines (EC_{50} < 2.5 nM) had a chloride at the 2 position on the benzene ring. Additional substitution of the benzene ring was also found to significantly affect the potency, although the position of the substitution is very important. For fludiazepam (EC_{50} = 7.04 nM), the addition of an additional fluorine at the 6 position on the benzene (difludiazepam) was found to increase the potency (EC_{50} = 2.97 nM). In comparison, the addition of a chloride to the 4 position was found to significantly reduce the potency, as seen with 4'chloro deschloroalprazolam.

Conclusion: Dose-response curves and potency data for at least 38 prescription and novel benzodiazepines detected on the illicit drugs market in Europe are reported in this study. This is the first report of the use of a cell-based $GABA_A$ receptor binding assay for benzodiazepines and the potency of novel benzodiazepines. There was large variation found in the potency of the benzodiazepines detected on the illicit market with results often differing from anecdotal data of dosing guidelines from user forums. Although potency is only one variable in determining dosage, this demonstrates the importance of analytically determining the potency of novel benzodiazepines, rather than relying on user reports.

Synthesis and functional evaluation of synthetic cannabinoid receptor agonists related to ADB-HEXINACA

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Background & Aims: ADB-HEXINACA has been recently reported as a synthetic cannabinoid receptor agonist (SCRA), one of the largest classes of new psychoactive substances (NPS). This compound marks the entry of the *n*-hexyl tail group into the SCRA landscape. This modification had not been observed previously, and has continued to appear, combined with both older and novel core scaffolds. Therefore, a proactive characterization campaign was undertaken, including the synthesis, characterization, and pharmacological evaluation of ADB-HEXINACA and a library of 41 closely related analogues. Both the novel *n*-hexyl and terminal *n*-hexenyl tail groups were examined across the indole, indazole and 7azaindole scaffolds, with a series of typical head groups, to comprehensively explore the chemical space. In addition, computational modelling studies and induced fit docking were carried out to rationalize key structure-activity relationships (SARs) observed in the series.

Methods: Two *in vitro* functional assays, based on a different ligand-induced signaling event, were employed to assess activity at CB_1 and CB_2 cannabinoid receptors. The membrane potential assay (MPA) measures the change in membrane potential resulting from $G_{\beta\gamma}$-coupled activation of inwardly rectifying potassium channels (GIRKs), leading to hyperpolarization of the cellular membrane. Additionally, functional activity was assessed employing cell-based NanoBiT® assays (NanoLuc Binary Technology, Promega). The latter monitor the recruitment of a truncated β-arrestin 2 (βarr2) in HEK293T cells stably expressing CB_1 or CB_2. The activation of CB_1 or CB_2, fused to one inactive subunit of a split nanoluciferase enzyme, initializes the recruitment of βarr2, fused to the other inactive subunit. The proximity between these subunits leads to the restoration of the enzyme's activity, which generates a measurable bioluminescent signal upon addition of its substrate. The potential agonist activity of all compounds was evaluated via single-point screening (100 nM) at both CB_1 and CB_2. Subsequently, the *n*-hexyl-indazole derivatives and the ADB-bearing SCRAs, generally conferring strong CB_1 and CB_2 activity, were selected for further evaluation. Induced fit docking was used to rationalize SARs *in silico*, with key receptor binding interactions observed at both CB_1 and CB_2.

Results & Discussion: All synthesized SCRAs demonstrated agonist activity at both CB_1 and CB_2. Efficacy (E_{max}) and potency (EC_{50}) values were calculated relative to the reference CP55,940, and several key SARs were identified. ADB-HEXINACA was a potent ligand at both CB_1 (CB_1 MPA pEC_{50} = 7.87 ± 0.12 M; E_{max} = 124 ± 5%; βarr2 pEC_{50} = 8.27 ± 0.14 M; E_{max} = 793 ± 42.5) and CB_2 (CB_2 MPA pEC_{50} = 7.49 ± 0.10 nM; E_{max} = 103 ± 5; βarr2 pEC_{50} = 9.26 ± 0.19 nM; E_{max} = 101 ± 5.9). Generally, SCRAs featuring indazole cores were more potent than their indole analogues, which were, on their turn, more potent than, or equipotent to the 7-azaindole derivatives. Compounds bearing ADB, MDMB and CUMYL moieties were potent CB_1 and CB_2 agonists, while SCRAs with an APP head group had the lowest activity.

Conclusion: This study confirms that the recently detected SCRA ADB-HEXINACA and its related *n*-hexyl and terminal *n*-hexenyl tail-containing compounds were all able to activate CB_1 and CB_2, although to a different extent. These findings underscore the importance of monitoring these potentially emerging SCRAs, given their generally high potency and efficacy.

NPS O-15

Short- and long-term stability of synthetic cathinones and dihydro-metabolites in human whole blood and urine samples

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Background & Aims: Synthetic cathinones, have become one of the most prevalent drug classes of new psychoactive substances (NPS) for their psychostimulant and euphoric effects. The concentration of analytes present in biological samples at the time of analysis may significantly differ from the time of sample collection. This is the case for synthetic cathinones as their stability is highly susceptible to degradation and thus, the stability of data of these drugs is crucial. Various factors may influence the stability of synthetic cathinones between sampling and analysis, and therefore, stability studies are required to determine the best storage conditions as well as extend the period of detection. With respect to the latter, it maybe helpful to investigate the potential biomarkers that can prove better stability in biological samples. To date, however, no comprehensive evaluation on the stability of synthetic cathinone metabolites in the biological samples has been published.

The purpose of this study was to investigate the stability of a panel of synthetic cathinones and dihydro-metabolites in human whole blood and urine samples under various conditions used in forensic toxicology using a validated liquid chromatography–tandem mass spectrometry method.

Methods: The validated methods (in both whole blood and urine) were used to assess the stability of 16 synthetic cathinones and 10 selected dihydro-metabolites, examining the effects that short and prolonged storage had on analyte stability. The whole blood samples were stored at room temperature, 4 °C, −20 °C, −40 °C for up to 6 months, while urine samples were stored either at room temperature (22–23 °C) for up to 3 days, refrigerated (4 °C) for up to 14 days or frozen (−40 °C) for up to 12 months. The influence of 2% sodium fluoride as a preservative in blood collection tubes was also investigated.

Results & Discussion: In whole blood and urine samples, instability was observed under all storage temperatures, but at a lower rate when stored frozen. Among frozen conditions, all parent drugs and dihydro-metabolites in whole blood samples demonstrated higher stability at − 40 °C than at − 20 °C. The effect of preservatives (2% sodium fluoride) was considered insignificant since minor/no changes were observed between preserved and unpreserved blood samples, except for room temperature. The stability differences within synthetic cathinone classes were diverse, in which analytes containing halogens were characterised by poor stability. Moreover, degradation of dihydro-metabolites was significantly slower than their parent drugs under all of the storage conditions. For instance, with the complete degradation of certain parent synthetic cathinone in whole blood especially at room temperature and 4 °C, there was no sampling point and storage condition where dihydro-metabolites were undetectable over the studied period. Concerning urine samples, unlike parent drugs, all dihydro-metabolites were fairly stable (>80% remaining) after 3 days and 14 days at room temperature and 4 °C, respectively. The only exception was for the halogenated metabolites that had more than 60% remaining under both conditions. Nevertheless, in comparison to their respective parent drugs, the reduced metabolites were overall more stable, making them more suitable biomarkers of synthetic cathinone use.

Conclusion: The data suggest that samples containing synthetic cathinones should be analysed immediately, if possible. Alternatively, whole blood and urine samples should be stored frozen (at − 40 °C or lower); however, (quantitative) results should be interpreted with caution after long-term storage. The data also promote the use of dihydro-metabolites as biomarkers for synthetic cathinones intake, as these reduced metabolites may be detected for longer period of time when compared with parent drugs in whole blood and urine samples.

Metabolism study of 3-chloromethcathinone (3-CMC) by dried blood spot (DBS) sampling after controlled administration using a murine model

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Background & Aims: Since the widespread diffusion of new psychoactive substances (NPS), forensic laboratories are often required to identify new drugs and their metabolites. In recent decades, an increasing number of NPS made their appearance on the market; among these, Synthetic Cathinones are the second largest category monitored by the European Early Warning System.

The use of micro-extraction techniques is becoming increasingly widespread, allowing the use of small volumes of sample and solvents while maintaining a high specificity and sensitivity for the detection of NPS in biological specimens.

The present study is aimed at the metabolism study of the synthetic cathinone 3-chloromethcatinone (3-CMC) in a murine model, using the Dried Blood Spot (DBS) technique for the sampling, storage, and purification of blood samples after controlled administration, and Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) techniques for the identification of metabolites and quantification of the parent drug.

Additionally, an evaluation of the stability of 3-CMC and its metabolites in DBS was conducted on authentic post-administration samples, elucidating the detectability of 3-CMC and/or its metabolites over a 90-day period.

Methods: Twenty-one female ICR (CD-1®) mice were injected with a dose of the synthetic cathinone and seven blood collection were performed at different times within a 24-hour period after the administration of 3-CMC. Each blood drop was collected at 1-2-4-6-8-12-24 hours after administration and deposited on the cartridge, left to dry at room temperature and analyzed afterwards. Each dried spot was extracted with 0.1% formic acid methanol, added with internal standard and subsequently the supernatant was evaporated to dryness and reconstituted in 100 µL of 0.1% formic acid water and 10 µL were directly injected in the LC-MS systems.

The samples were firstly analyzed by LC–HRMS for metabolites fragmentation pattern identification, using a benchtop Orbitrap Exactive mass detector. Subsequently, a LC-MS/MS method was developed for their identification and 3-CMC quantification in routine workload, using a Waters Acquity UHPLC system coupled to a triple quadrupole mass spectrometer Xevo TQ-S micro operating in Multiple Reaction Monitoring. LC-MS/MS method was fully validated within a previous interlaboratory study about stability of synthetic cathinones in DBS [1].

Additional DBS samples, collected as the previous, were deposited, stored at room temperature, and analyzed at a later stage to evaluate the stability of 3-CMC and its metabolites 30, 50 and 90 days after first quantification.

Results & Discussion: The LC–HRMS analyses performed on dried blood samples identified three metabolites in all the mice specimens, besides detectable amounts of the unchanged drug within the first 6 hours after administration. The main metabolic reactions observed were reduction of the β-keto group, N-demethylation and a combination of both, in accordance with other studies. A double peak was detectable for the dihydro-CMC metabolite, which is consistent with its two stereoisomers.

The area ratios of 3-CMC and its metabolites/ISTD show the decrease of concentration of 3-CMC and the contemporaneous increase of the metabolites within 8 hours post administration; the detection of metabolites extends the time window of detectability of CMC intake even after the parent drug is eliminated by the bloodstream.

Stability, obtained in terms of mean percentage deviation from the initial concentrations, was also evaluated for 3-CMC and its metabolites.

As already mentioned in the literature, the main drawback of the analysis of cathinones is their high degree of instability, due mostly to the nature of the molecule but also, to a lesser extent, to the storage conditions, which can hinder their detection in biological samples. The choice of dried blood spots as storage medium helps mitigating this stability issue; moreover, the presence of the hydroxy group in the metabolites (percentage decrease 5%-37%) appears to increase the stability of the analyte when compared to the parent (67%-82%).

Furthermore, the use of DBS minimizes the volume of sample collection and the problems associated with storage, transport and health hazards of blood specimens.

Conclusion: The present application of DBS on authentic samples allows the identification and quantification of 3-CMC and its metabolites with the use of small volumes of samples while maintaining a high specificity and sensitivity.

The metabolite profile of the synthetic cathinone 3-CMC was studied by analyzing blood samples collected within 24 hours after a controlled administration and allowed the identification of three metabolites (dihydro-CMC, N-demethyl-CMC, dihydro-N-demethyl-CMC). DBS demonstrated themselves to be a versatile, rapid, and more environmentally sustainable sample storage and pre-treatment technique, and appeared to be suitable for the analysis of the synthetic cathinone 3-CMC in blood samples to evaluate the stability of the parent drug and its metabolites.

[1] Morini L, Odoardi S, Mestria S, et al (2024). Microchem J 200:110394.

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NPS O–17

3-CMC, 4-CMC, and 4-BMC metabolic profiling in humans: New major metabolic pathways to document consumption of methcathinone analogues

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Background & Aims: Synthetic cathinones represent one of the largest and most abused novel psychoactive substance (NPS) classes, and have been involved in many acute intoxications and fatalities worldwide. Methcathinone analogues such as 3-methylmethcathinone (3-MMC), 3-chloromethcathinone (3-CMC), and 4-CMC (clephedrone) currently constitute most of synthetic cathinone seizures in Europe (>95% in 2021, synthetic cathinones constituting 50% of total NPS seizures). Documenting their consumption in clinical and forensic casework is therefore essential to tackle this trend. Targeting metabolite biomarkers is a go-to to prove synthetic cathinone consumption in analytical toxicology, and metabolite profiling is crucial to support investigations. In this study, we aimed at assessing the metabolic profile of methcathinone analogues 3-CMC, 4-CMC, and 4-bromomethcathinone (4-BMC, brephedrone) in humans to identify specific metabolite biomarkers of consumption.

Methods: 3-CMC, 4-CMC, 4-BMC, and positive and negative controls were incubated with cryopreserved 10-donor-pooled human hepatocytes for 0 and 3 h. The incubates were analyzed by liquid chromatography-high-resolution tandem mass spectrometry (LC-HRMS/MS) with a Q Exactive (Thermo Scientific); LC separation was performed through a biphenyl-bonded column (150 × 2.1 mm, 2.6 µm; Phenomenex) with a mobile phase gradient composed of 0.1% formic acid in water and 0.1% formic acid in acetonitrile, and MS detection was performed in full-scan/data-dependent MS/MS mode after electrospray ionization in positive and negative modes. Raw LC-HRMS/MS data was mined with Compound Discoverer (Themo Scientific) for an exhaustive screening. 3-CMC-positive blood and urine (case #1), 3- and 4-CMC-positive oral fluid (case #2), and 4-CMC-positive urine (case #3) from clinical or forensic casework were analyzed with the same workflow. The analysis was supported by human metabolite predictions with GLORYx freeware (University of Hamburg, Germany).

Results & Discussion: A total of 9, 9, and 10 metabolites were identified in 3-CMC, 4-CMC, and 4-BMC incubates, respectively; 3 and 2 additional minor phase II metabolites were found in 3- and 4-CMC-positive samples, respectively. Good correlation was found between in vitro and in vivo results, confirming that human hepatocyte incubation is a good model for predicting synthetic cathinone metabolism. The same transformations occurred for the three substances, major reactions including β-ketoreduction and *N*-demethylation, as expected from the data reported on the metabolism of other analogues. More surprisingly, predominant metabolites in hepatocyte incubations but also in positive blood and urine samples were produced by combination of *N*-demethylation and ω-carboxylation (most intense metabolite in 4-CMC-positive case #3), and combination of β-ketoreduction, oxidative deamination, and *O*-glucuronidation (most intense metabolite in 3-CMC-positive case #1). These latter phase II metabolites were detected in negative-ionization mode only, and their non-conjugated form was not detected with MS after glucuronide hydrolysis, likely due to the lack of a chemical group susceptible to ionization; this metabolic pathway was never reported for 3-CMC, 4-CMC, and other popular methcathinone analogues susceptible to undergo the same transformations such as 3- and 4-MMC (mephedrone). 3-CMC, 4-CMC, and 4-BMC metabolites formed by β-ketoreduction (urine and blood); combination of *N*-demethylation and ω-carboxylation (urine and blood); and combination of β-ketoreduction, oxidative deamination, and *O*-glucuronidation (urine) are recommended as biomarkers of consumption, along with the parent drug. Glucuronide hydrolysis of urine samples is not advised.

Conclusion: New major metabolic pathways were identified for 3- and 4-CMC in humans, and 4-BMC metabolism was studied for the first time. Further investigation is needed to understand whether metabolites combining

β-ketoreduction, oxidative deamination, and *O*-glucuronidation are also major with other methcathinone analogues and, more broadly, other synthetic cathinones depending on the amine group substitutions. Further pharmacokinetic study is also needed to understand the relevance of the newly identified metabolites to document early and late consumption. These results strongly support the need for comprehensive screening strategies in metabolite identification studies, to avoid overlooking significant metabolites and major biomarkers of consumption.

Assessment of α-PVP disposition in oral fluid, sweat and urine in humans by GC-MS/MS analysis

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Background & Aims: α-PVP belongs to the fourth generation of synthetic cathinones and it is structurally related to pyrovalerone. Several cases of intoxication and deaths due to α-PVP consumption alone or in combination with other substances have been reported in the literature. The substance is still used in recreational settings with different routes of administration (oral, intranasal, intravenous, rectal) even if since 2016, it is controlled in the majority of EU countries and is scheduled in the United Nations Convention on Psychotropic Substances of 1971. This observational nauturalistic study in humans aimed for the first time to investigate α-PVP disposition in oral fluid (OF), its excretion in urine and sweat (SW) of consumers through GC-MS/MS analysis.

Methods: Nine healthy psychostimulants users (3 women, six men) were enrolled in the study, provided a signed informed consent and were required a wash-out period before the substance administration . Six volunteers self-administered intranasal 20mg α-PVP, while the others three 10mg. OF samples were collected before and at 0.33, 0.66, 1, 1.5, 2, 2.5, 3, 4 and 5h after α-PVP intake. Pooled urine samples were collected between 0-2h and 2-5h after α-PVP intake. For every volunteer, two SW patches were collected, 4h prior to the administration (-4-0h) and up to 5h (0-5h) following consumption. An amount of 50µL OF and urine samples were liquid-liquid extracted with ethylacetate, after the addition of IS (α-PVP-d_8) and 100µL of 0.5M ammonium hydrogen carbonate, dried gently under nitrogen stream and reconstituted in 50µL ethylacetate. Patches were pretreated by removing the absorbent pad, then fortified with IS and sonicated in methanol for 1h. Finally, 100µL was dried under nitrogen stream and reconstituted in 50µL ethylacetate. Analyses were performed on 7890B GC system coupled to 7000C triple quadruple mass spectrometer operating in multiple reaction monitoring mode.

Results & Discussion: α-PVP OF concentration reached a median peak of 2.6µg/mL (range 0.58-16µg/mL) at a median T_{max} of 0.83h (range 0.33-1.0h) following 20mg dose, while after 10mg single dose it peaked at a median T_{max} of 1.0h with a median C_{max} of 1.0µg/mL (range 0.11-1.1µg/mL). α-PVP was detectable in OF at the final 5h time point in 8 out of 9 participants. α-PVP and its most abundant metabolite formed by reduction of the ketone structure were determined in urine, the median α-PVP total amount excreted in 5h was 89 and 108µg after intranasal doses of 20 and 10mg, respectively. Interestingly, 5h post administration only 2 patches out of 6 volunteers who snorted a single dose of 20mg, were found to contain α-PVP with concentrations of 31.7 and 64.5 ng/patch. Conversely, α-PVP was excreted in sweat at concentrations of 18.3, 24.8 and 1477ng/patch in consumers snorting 10mg.

Conclusion: To the best of our knowledge, our results provide for the first time information about the disposition in humans of α-PVP in OF and its excretion in urine and sweat after a controlled intranasal administration. Our results showed that α-PVP is rapidly absorbed, within the first hour, at both doses. Additionally, intranasal doses of 20 and 10mg resulted in excretion rates of the parent compound of only 0.45% and 1.1% in 5 hours. Despite the small sample size and controversial results, this preliminary study revealed that α-PVP excretion occurs also in sweat.

Scientific Session 13 – Free topics

14:00 – 15:00 Friday, 6th September, 2024

Chair: Barry Logan, Jean-Claude Alvares

DIV O-1

Epidemiological profile of pregabalin misuse and abuse in the region of Ouargla, Algeria

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Background & Aims: Pregabalin (PGB) misuse represents a growing trend in worldwide, leading to dependence and acute poisoning with mortality risk. There is a lack of data in on PGB misuse and abuse in Algeria. Thus, the present study aimed to determine its local epidemiological characteristics

Methods: An observational retrospective study was conducted over a period of seven years from January 1st, 2017, to October 31st, 2023. The included population were patients admitted for PGB misuse and poisoning, in adult and paediatric emergencies departments of public health establishments. Statistical analysis and data visualization were performed using R software.

Results & Discussion: During the last seven years (2017-2023), 152 cases of PGB misuse and poisoning were recorded, representing 10.78% of acute poisoning cases. A significant increase in the incidence of PGB poisoning cases has been observed, ranging from 0.84% in 2017, to 15.54% in 2022, then 21.46% in 2023. Patients mean age was 24±8-year-olds, and 90.78% were male. Urine analysis remained positive for only PGB in 35.52% of cases, and PGB combined with other psychoactive substances (64.48%) including cannabis (50.65%), benzodiazepines (23.68%), MDMA (15.13%), amphetamines (12.5%), tramadol (4.6%), cocaine (3.94%), illicit opioids (2.63%), tricyclic antidepressants (1.97%), barbiturates (1.97%), buprenorphine (1.31%), and alcohol (1.31%). Recorded patients have presented various neurological signs including: disorders of consciousness (46.71%), drowsiness (30.92%), psychomotor agitation (11.18%), coma (3.94%), miosis (3.94%), anxiety (3.94%), convulsions (3.28%), and altered mental status (2.64%). The evolution was favourable in 96.72% of the cases. The lethality rate was calculated as 3.28%.

Conclusion: PGB misuse and abuse's incidence has increased in the last two years in the region of Ouargla, and is becoming a public health issue. Awareness campaigns are recommended.

DIV O-2

Structure-activity relationship of P-gp ATPase modification by synthetic cannabinoids and drug-drug interaction prediction

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Background & Aims: The human P-glycoprotein (P-gp) as prominent efflux transporter has strong impact on drug pharmacokinetics. P-gp is involved in drug disposition due to its presence in the intestine, the blood-brain barrier, as well as in excretory tissues. Relevant drug-drug interactions can occur if the transport and in turn the distribution of a drug is increased or decreased by a second compound. Unfortunately, very limited information about the influence of drugs of abuse and especially new psychoactive substances (NPS) on P-gp activity is available. The aims of the present study were thus to investigate a total of 20 synthetic cannabinoids (SC) with different structural properties for their potential to modify in vitro P-gp ATPase activity. Findings may allow to elaborate a structure-activity relationship and predict potential drug-drug interactions.

Methods: Membranes with cDNA-expressed, human P-gp were incubated with three different concentrations (2, 20, 200 µM, n = 3) of each SC (A-CHMINACA, A-FUBINACA, 3,5-AB-5F-FUPPYCA, AB-5F-P7AICA, AB-CHMFUPPYCA, ADB-BINACA, Cumyl-4CN-BINACA, DMBA-CHMINACA, MDMB-4en-PINACA, MDMB-4F-BINACA, MDMB-5F-PICA, MDMB-FUBICA, MMB-4en-PICA, MMB-CHMICA, MMB-FUBINACA, MPhP-5F-PICA, 2F-QMPSB, QMMSB, QMPCB, or SGT-233) in presence of adenosine 5'-triphosphate (ATP, 4 mM) at 37°C for 20 min. Additional coincubations of each SC (2, 20, 200 µM, n = 3) with verapamil (20 µM) were conducted. ADP formation was measured and quantified by hydrophilic interaction liquid chromatography-high-resolution tandem mass spectrometry in negative ionization mode and compared to reference incubations, such as incubations without P-gp or with the recommended P-gp model substrate verapamil. A one-way ANOVA followed by Dunnett's multiple comparison test was used to test for statistically significant differences.

Results & Discussion: As ATP hydrolysis followed by release of adenosine 5'-diphosphate (ADP) and inorganic phosphate is crucial for P-gp transport, ADP formation was measured and used as in vitro marker for P-gp AT-

Pase activity. Such an increased activity often correlates with in vivo P-gp transport activity. Sixteen out of 20 SC showed statistically significant P-gp ATPase stimulation. Fifteen of them increased the ADP formation at the lowest test concentration of 2 µM indicating high affinity to the P-gp ATPase. In case of 10 SC, the ADP formation was higher compared to that in reference incubations with verapamil. In terms of the structure-activity relationship, the head group seemed to be the major determinant of the ATPase stimulation ability of a SC. Head group derived from adamantane, or the amino acids valine or phenylalanine were beneficial, but not those derived from *tert*-leucine. The structures of core, linker, and tail seemed to have minor influence on the P-gp ATPase affinity of the SC. Coincubations of the SC with verapamil were used to test for possible in vivo drug-drug interactions. Eight out of 20 SC showed statistically significant reduced ADP formation compared to reference incubations with verapamil alone. Five of them decreased the ADP formation at the lowest test concentration of 2 µM. Reduced ADP formation in coincubations may indicate an inhibition of the P-gp followed by reduced transport capacity. P-gp membranes are limited in their prediction potential because they only indicate P-gp ATPase activity but not the transport through the cell membrane. However, membrane-based experiments can be a good starting point for follow-up experiments using vesicular- or cell layer-models.

Conclusion: Membrane-based experiments represent a rapid P-gp ATPase stimulation screening tool. The presented data demonstrate that many SC interact with P-gp and that the structure of the head group is of particular importance. Some SC were shown to inhibit the P-gp ATPase in coincubations. A thorough monitoring of the toxicokinetics of NPS is crucial for risk assessment and prevention and further characterization of potential P-gp substrates or inhibitors among the SC must be accelerated.

Amino acids and their metabolites as biochemical markers of putrefaction

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DIV O-3

Background & Aims: *Post mortem* decay is influenced by several processes, especially autolysis, the degradation of macromolecules such as proteins by hydrolytic enzymes, and putrefaction, the (further) microbial metabolism/degradation of the aforementioned compounds and autolysis end products e.g., amino acids. Amino acids can be metabolized by bacterial enzymes leading to decarboxylation, *N*-acetylation, hydroxylation, and/or transamination/deamination. The aim of this study was to analyze amino acids and their bacterial degradation products/metabolites by liquid chromatography – high-resolution mass spectrometry (LC-HRMS) in a semiquantitative way, to investigate their potential as an indicator of the putrefaction grade. For this purpose, the selected amino acids and metabolites were monitored in vitreous humor from pig eyes over a study period of four days.

Methods: Sixty pig eyes were freshly obtained from a local butcher and stored on either petri dishes or truncated self-standing conical centrifuge tubes (n=30, each) within a climatic chamber at 25°C and 65% humidity for up to four days. Every day vitreous humor was drawn from six eyes, respectively, and frozen at -80°C. For sample preparation, 10 µL of vitreous humor were precipitated by 50 µL of a acetonitrile-methanol-mixture (4:1, *v:v*). Afterwards, the supernatant was diluted 1:4 (*v:v*) by mobile phase A (10 mM ammonium formate, 0.1% formic acid) and analyzed by LC-HRMS^n operated in fullscan MS^1 and MS^2 detection modes. Amino acids and their metabolites were identified by comparison of the obtained HRMS^2 spectra to those of purchased analytical standards, reference spectra databases, careful fragmentation pattern analysis, and/or *in silico* structure prediction. For semiquantitative analysis peak areas were analyzed in fullscan mode.

Results & Discussion: Five amino acids (lysine, arginine, tyrosine, phenylalanine, tryptophan) and ten amino acid metabolites (N6-acetyllysine, N-acetylcadaverine, N-acetylagmatine, tyramine, 2-phenylethylamine, kynurenic acid, N-acetyltyramine, N-acetyltryptophan, N-acetyl-2-phenylethylamine, N-acetyltryptamine) were detected and (putatively) identified in vitreous humor. All five investigated amino acids were already detected from day 0. Especially the aromatic amino acids, such as phenylalanine, showed a significant increase from day 0 to day 4 of up to 46-fold, while for arginine no trend was found. Observed increases in amino acid peak areas are most likely due to proteolysis by hydrolytic enzymes leaking from degraded cells during autolysis. The amino acid metabolites including acetylated amino acids, biogenic amines, acetylated biogenic amines, and others were not present on day 0. Most of these compounds were first detected on day 2 and peak areas were strongly increased by days 3 and 4, suggesting that these compounds were formed during putrefaction processes. In particular, for the amino acid metabolites N6-acetyllysine, N-acetylcadaverine, kynurenic acid, N-acetyltyramine, and N-acetyltryptophan significant increases over time were observed (t-test, α=0.05). However, high variability was found among the six eyes within the same condition (type and time of storage). This was especially true for N-acetylagmatine, tyramine, 2-phenylethylamine, and N-acetyl-2-phenylethylamine. Comparison of the two storage conditions on either truncated conical centrifuge tubes or petri dishes showed no significant differences (t-test, α=0.05) with respect

to peak areas of the investigated analytes, except for arginine (slightly higher in conical centrifuge tubes on day 2, markedly higher in petri dishes on day 3) and N6-acetyllysine (markedly higher in conical centrifuge tubes on day 3).

Conclusion: Amino acids and their metabolites could serve as potential markers to characterize the putrefaction process and thus as an estimation tool for the *post mortem* interval. Particularly, aromatic amino acids and their metabolites seem to be candidates for this purpose. However, further investigation is needed to identify influencing factors on amino acid/amino acid metabolite profiles and involved microbes.

DIV O–4

Mitragynine and 7-hydroxymitragynine plasma pharmacokinetics after controlled oral mitragynine isolate administration to healthy human subjects

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Background & Aims: There currently are no FDA or EMA approved uses for kratom or mitragynine, its primary active alkaloid. Tremendous growth in kratom research, primarily funded by the National Institute on Drug Abuse, highlight mitragynine's unique mechanisms of action including pain relief due to partial agonism at the μ-opioid receptor without recruiting the beta-arrestin pathway thought to be responsible for respiratory depression, side-effects of prototypical opioids. Last year we provided pharmacokinetic data after controlled single and 15 consecutive multiple doses of dried oral kratom leaf doses in capsules up to 4000 mg or 53.2 mg mitragynine to healthy adults to improve understanding of mitragynine and its active metabolite, 7-hydroxymitragynine (7-OH-MTG) concentrations (Huestis et al *Molecules* 2024). This year, we present pharmacokinetic data from the first placebo-controlled, randomized, escalating dose human administration study of mitragynine isolate (Mitra-Isolate™).

Methods: A placebo-controlled, ascending single and 15 consecutive daily doses of mitragynine isolate (11.15, 22.3, 44.6 and 89.2 mg in capsules) was conducted in 48 healthy individuals during 31 in-person visits with plasma sampling up to 10 and 23 days after single and multiple doses, respectively. Participants were kratom naïve or had not used kratom in at least 12 months. The Advarra Institutional Review Board and Health Canada approved the study. Informed consent was obtained from all subjects involved in the study. Mitragynine and 7-OH-MTG concentrations were determined by a fully-validated LC-MS/MS method according to the FDA Guidance for Industry and EMA Guideline on Bioanalytical Method Validation. Sample pretreatment consisted of protein precipitation of 100 μL plasma after addition of mitragynine-D3 and 7-OH-MTG-D3 internal standards. Linearity was 0.5–500 ng/mL for mitragynine and 0.5-100 ng/mL for 7-OH-MTG. Between-run accuracy (% bias) was -0.6-2.2% and 2.4-10.4% and between-run precision (%CV) 3.9-9.3% and 4.2-10.4% for mitragynine and 7-OH-MTG, respectively. Four quality control samples spread across the linear range for each analyte were included in each batch.

Results & Discussion: Table 1 Median (range) C_{max}, AUC_{0-24} h*ng/mL and T1/2 for mitragynine and 7-OH-MTG after single (SD) and 15 multiple (MD) mitragynine isolate doses.

Mitra-isolate™	11.15 mg	22.3 mg	44.6 mg	89.2 mg
SD	**C_{max} ng/mL**			
n	11	12	12	12
Mitragynine	30.5 (9.1-72.4)	77.0 (30.8-142)	124 (51.1-494)	219 (105-445)
7-OH-MTG	5.1 (3.1-11.7)	14.1 (6.9-23.2)	22.5 (11.8-59.7)	34.2 (16.2-60.9)
		AUC_{0-24} h*ng/mL		
Mitragynine	92.3 (23.2-144)	248 (69.2-425)	490 (238-1244)	806 (432-1937)
7-OH-MTG	12.7 (8.5-36.4)	48.3 (22.7-85.1)	102 (41.1-231)	163 (88.5-307)
		T1/2 h		
Mitragynine	12.9 (1.5-53.4)	21.0 (1.4-65.0)	51.2 (25.4-94.6)	42.9 (17.8-80.0)
7-OH-MTG	1.8 (0.9-4.0)	3.2 (1.3-8.1)	4.6 (2.7-24.8)	4.0 (2.7-24.0)
MD		**$C_{max\ steady\ state}$ ng/mL**		
n	11	10	12	8
Mitragynine	38.2 (14.8-108)	111 (43.7-160)	174 (73.5-425)	336 (193-753)
7-OH-MTG	6.1 (4-14.4)	13.6 (7.2-21.5)	21.6 (10.3-46.2	42.6 (28.9-63.3)
		$AUC_{0-Tau\ Steady\ state}$ h*ng/mL		
Mitragynine	124 (45.1-259)	437 (136-806)	923 (342-1904)	1675 (848-2730)
7-OH-MTG	15.6 (10.2-41.1)	55.5 (20.8-135)	125 (37.7-247)	248 (112- 354)
		$T1/2_{\ Steady\ state}$ h		
Mitragynine	50.8 (4.2-88.4)	45.8 (17.9-98.5)	64.7 (30.5-104)	44.5 (42.6-78.6)
7-OH-MTG	2.8 (1.5-5.0)	4.1 (1.7-64.6)	30.6 (2.5-129)	33.7 (4.3-135)

There were no serious adverse events or deaths, and the participant with the highest mitragynine concentration (753 ng/mL) completed the study. Mitragynine median T_{max} ranged from 0.75-1.2 h after a SD and from 0.75-1.2 h after 15 MD. 7-OH-MTG median T_{max} were similar from 1.0-1.5 h after a SD and 1.0-1.7 h after 15 MD. Dose proportionality was assessed on the exposure parameters C_{max} and AUC for mitragynine and 7-OH-MTG after SD and MD using a power model. C_{max} for both analytes after SD and MD fulfilled the condition for dose proportionality. $AUC_{0-Tlast}$ (SD) and $AUC_{0-tau,ss}$ (MD) increased slightly higher than dose proportional for mitragynine SD and MD and for 7-OH-MTG MD, with dose proportionality shown for $AUC_{0-Tlast}$ of 7-OH-MTG after SD. Steady state for mitragynine and 7-OH-MTG was reached between 7 and 10 days after the start of MD. Mean mitragynine accumulation factors across the four doses after MD were between 1.3 - 1.7 for C_{max} and between 1.7 - 1.8 for AUC_{0-24} and for 7-OH-MTG 1.1 -1.2 for C_{max} and between 1.2 -1.3 for AUC_{0-24}.

Conclusion: This mitragynine isolate human study greatly extends the controlled administered dose range to 89.3 mg mitragynine and provides the first human data on mitragynine isolate pharmacokinetics. Overall, all doses were well tolerated and no serious adverse events were reported. Maximum individual mitragynine concentrations up to 753 ng/mL were observed at steady state in the present study. The current study provides critically needed pharmacokinetic data for interpreting potentially therapeutic and toxic mitragynine and 7-OH-MTG concentrations.

Characterization of 'prophetic' nitazenes: insights from *in vitro* μ-opioid receptor assays and *in vivo* behavioral studies in mice

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Background & Aims: New synthetic opioids (NSOs) continue to contribute to drug-related fatalities worldwide. Since 2019, various NSOs with a 2-benzylbenzimidazole scaffold ('nitazenes') have been detected in the recreational drug market, with six nitazenes identified for the first time in Europe in 2023. While new to the recreational drug market,

different nitazenes (e.g., isotonitazene, etazene) were originally studied in the 1950s-60s for their potential as analgesics. Others, such as *N*-pyrrolidino protonitazene (protonitazepyne), have never been evaluated before, and are truly 'new' synthetic opioids. As the group of nitazenes continues to expand, so does the diversity and complexity of various analogues at the street level, posing an increasingly significant threat to public health worldwide. Here, we synthesized and characterized 15 nitazenes differing in 4 positions of the 2-benzylbenzimidazole core structure (R_1-R_4). These 'prophetic' analogues were selected from old literature (*n* = 8) or newly designed (*n* = 7) based on their predicted biological activity and relative ease of synthesis. The results aim to expand the existing knowledge about 2-benzylbenzimidazole structure-activity relationships (SAR), while also helping stakeholders (e.g., forensic toxicologists, clinicians, policymakers) in their risk assessment and preparedness for the potential next generation of nitazenes.

Methods: *In vitro* characterization focused on the μ-opioid receptor (MOR), the primary molecular target for clinically applied and abused opioids. MOR affinity was determined via competition radioligand (^{3}[H]DAMGO) binding assays in rat brain tissue. MOR activation (potency and efficacy) was studied by means of a cell-based β-arrestin 2 (βarr2) recruitment assay. To complement *in vitro* findings, *in vivo* experiments were performed to investigate opioid-like effects in male C57BL/6J mice for seven analogues of the highly potent etonitazene.

Results & Discussion: Radioligand binding assays revealed that all nitazenes bind to MOR with nanomolar affinities (K_i=8-431 nM). In the βarr2 recruitment assay, all compounds activated MOR with potencies ranging from 0.588 nM (etonitazene) to 1266 nM (ethylene nitazene). Methionitazene (EC_{50}=5.28 nM) and α'-methyl etonitazene (EC_{50}=1.00 nM) were the only newly studied analogues with a higher potency than fentanyl (EC_{50}=17.0 nM). While most newly studied nitazenes were less active than their corresponding comparator 2-benzylbenzimidazole, notable exceptions were iso-butonitazene (EC_{50}=10.3 nM) and sec-butonitazene (EC_{50}=7.62 nM), the potencies of which exceeded that of butonitazene (EC_{50}=34.2 nM). Furthermore, methylnitazene (EC_{50}=70.2 nM) and propylnitazene (EC_{50}=9.50 nM) were more potent than nitazene (EC_{50}=312 nM). Efficacies ranged from 187-254% (compared to hydromorphone) in the employed assay; 1-ethyl-pyrrolidinylmethyl *N*-desalkyl etonitazene (E_{max}=173%), 5-trifluoromethyl isotodesnitazene (E_{max}=133%), and nitazene (E_{max}=172%) were the only analogues with a lower efficacy than fentanyl (E_{max}=209%). *In vivo*, dose-dependent effects were observed for antinociception, locomotor activity, and body temperature changes in mice. The antinociceptive potency of etonitazene was 0.022 mg/kg. The most and least potent analogues were α'-methyl etonitazene (ED_{50}=0.060 mg/kg) and ethyleneoxynitazene (ED_{50}=11 mg/kg), respectively, and all analogues reached the maximum level of antinociception (i.e., 45-second cut-off). Bell-shaped curves were obtained for locomotor activity. The maximum distance traveled by the animals was largely comparable between the different treatment groups (~320-410 m), and all analogues induced a comparable maximum decrease in body temperature compared to baseline (2.0-2.6°C).

Taken together, various new SAR insights were gained. At R_1 (*para*-benzyl), ethoxy and isopropoxy substituents remain the "sweet spot" in terms of opioid activity, and corresponding thioalkyl analogues (e.g., methionitazene) may be equally active. Analogues with branched alkoxy tails (e.g., iso-butonitazene, sec-butonitazene) showed an increased activity compared to compounds with longer linear tails, whereas decreased potencies were observed in analogues with trifluoromethyl (e.g., trifluorometonitazene) and alkyl (e.g., propylnitazene) substitutions. A rigid dihydrofuran moiety (e.g., ethyleneoxynitazene) conferred decreased *in vitro* and *in vivo* opioid activity. At R_2, the importance of the 5-nitro group was further confirmed, with 5-methyl (e.g., 5-methyl etodesnitazene) and (particularly) 5-trifluoromethyl (e.g., 5-trifluoromethyl isotodesnitazene) substitutions leading to a decreased *in vitro* opioid activity. Modification of R_3 by appending a 1-ethyl-pyrrolidinylmethyl group at the benzimidazole N1-atom (i.e., 1-ethyl-pyrrolidinylmethyl *N*-desalkyl etonitazene) reduced *in vitro* and *in vivo* opioid activity compared to a diethylamino chain. At R_4, lengthening of the methylene bridge between the benzimidazole and benzyl moieties substantially decreased opioid activity (e.g., ethylene etonitazene, ethylene nitazene). Importantly, α'-methyl substituted 2-benzylbenzimidazoles (e.g., α'-methyl etonitazene) have the potential to yield highly active opioids.

Conclusion: As nitazenes are increasingly present at the street level – as individual drugs, mixed with or mis-sold as heroin or other drugs, or as ingredients of fake opioid, benzodiazepine or ecstasy tablets – close monitoring of market developments and rapid characterization of newly emerging analogues is of critical importance. Through careful pharmacological characterization, this study aims to inform stakeholders regarding nitazenes that have been actively contributing or might be anticipated to contribute to the nitazene as well as the larger opioid crisis.

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Poster gallery – NPS F-P-1 to P-51

10:00 - 10:30 Tuesday, 3rd September, 2024

NPS F-P-01

A comprehensive HPLC-MS/MS method for the detection of hexahydrocannabinol (HHC) epimers and related metabolic profile and its application to in vitro metabolic studies of stereochemically pure and mixture of HHC epimers

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Background & Aims: Hexahydrocannabinol (HHC) is a semi-synthetic cannabinoid that recently emerged in the dynamic landscape of novel psychoactive substances (NPS). It has been flagged by European Authorities for its concerning availability in the online market due to its similarities to Delta-9-tetrahydrocannabinol (Δ9-THC i.e., the main component of cannabis). To date, several research groups have explored the gap in knowledge related to human pharmacological or toxicological studies. However, since HHC exists as a mixture of two epimers, configured as 9(S)-HHC and 9(R)-HHC, particular attention needs to be posed by toxicologists to account for the impact of its epimeric composition in these studies. Therefore, the chromatographic separation of such epimers is a crucial point in the development of analytical methods to evaluate the human metabolic profiling of HHC and its epimeric composition. This research study provides (i) a comprehensive HPLC-MS/MS analytical method for the full separation of 9(S)- and 9(R)-HHC and the related metabolic profile in a single analytical chromatographic run and (ii) results from the in vitro metabolic studies of single incubated stereochemically pure HHC epimers and mixture.

Methods: An UPLC Acquity system (Waters) coupled with a Xevo TQ-S Triple Quadrupole Mass Spectrometer (HPLC-MS/MS, Waters) system was used and Multiple Reaction Monitoring (MRM) was employed in the positive ion mode (ES+). Chromatographic analysis was conducted with a HSS T3 column (100Å, 1.8 µm, 2.1 mm x 150 mm) (Acquity, Waters). Several conditions were studied for the optimisation of the chromatographic method. Isocratic conditions by using a mixture of water, methanol and 0.3% formic acid (20:80:0.1 v/v) enabled attaining effective chromatographic separation of 9(S)-HHC and 9(R)-HHC (crucial for the in vitro experiment) and related metabolites. For the in vitro metabolism using pooled Human Liver Microsomes (HLM), three sampling points were collected to monitor the experiment: at time zero (T0), 90 minutes (T90), and 180 minutes (T180). Single epimer 9(S)- HHC and 9(R)-HHC) and the mixture 9(S/R)-HHC were incubated in duplicate at the final concentration of 50 µM. A total of 10 reactors were prepared, including adequate negative controls. Twenty µL of phosphate buffer of 50mM and 20 µL of either mixed substrate or single substrate was added to all reactors except for the blank with HLM and the blank with regenerating system. 2 µL of alamethicin solution at 12.5 mg/mL, and 20 µL of UDPGA solution 100 µM were added to all reactors except for the blank with only HLM and the blank of substrate mix with HLM. Ice-cold acetonitrile was added to each reactor at the end of each collection point to end the reaction. After 1.5 hours of incubation 20 µL of PAPS solution at 100 µM were added. All the samples were vortexed and centrifuged for 10 minutes at 1000 rpm. The supernatant was transferred and evaporated at 40°C. Samples were reconstituted in 50 µL of internal standard mixture at 100 ng/mL in H2O/MeOH (80:20) and transferred into a 250 µL glass vial with a fused insert for LC-MS/MS analysis.

Results & Discussion: The proposed analytical method via HPLC-MS/MS was developed to inform the in vitro study for the detection of HHC and its metabolic profile. The fully chromatographic separation of HHC epimers was achieved, including their isomeric metabolites, such as 11-nor-9(S)-carboxy-HHC, 11-nor-9(R)-carboxy-HHC, (+/-)-9α-hydroxy-HHC, (+/-)-9β-hydroxy HHC, 11-hydroxy-9(S)-HHC, and 11-hydroxy-9(R)-HHC. Experimental in vitro metabolic studies of stereochemically pure incubated epimers and mixture of HHC epimers by using HLM produced the formation of metabolites, such as 9β-hydroxy-HHC, 11-hydroxy-9R-HHC, 11-hydroxy-9S-HHC. This data confirmed the formation of the defined stereochemistry of hydroxy-metabolites in the reactor where the single epimer was incubated.

Conclusion: Detection and separation of the isomers 9(S)-HHC and 9(R)-HHC along with their metabolites were achieved by using an isocratic HPLC-MS/MS method. The metabolites 9β-hydroxy-HHC, 11-hydroxy-9R-HHC, were formed in the reactors where the epimer 9(R)-HHC was incubated, whilst 11-hydroxy-9S-HHC was from the 9(S)-HHC reactor. As a result, the analytical methodology could be applied to further research related to HHC in other matrices.

Metabolic biotransformation of synthetic cannabinoid receptor agonists (SCRAs)

NPS F-P-02

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Background & Aims: Synthetic Cannabinoid Receptor Agonists (SCRAs) are a sub-category of substances that belong to a group known as New Psychoactive Substances (NPS) and are often sprayed onto plant material, which is then smoked like Cannabis. These compounds act on the same CB1 and CB2 receptors that produce the effects when cannabinoids like delta-9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD) are used. A significant issue with SCRAs is that their chemical structures can be altered to enhance or change the desired effects, which is associated with a higher risk of adverse events occurring, including death. The designer nature of SCRAs has allowed them to easily circumvent the generic structure bans with slight modifications while retaining the ability to agonise the CB1 and CB2 receptors.

To predict metabolites that could be formed in the human system, human liver microsomes (HLM) can be used to break down a SCRA into its metabolites in an in-vitro model. The resulting metabolites could be potential biomarkers for detecting a SCRA within a human system. One drawback of using HLM is its capability to only produce Phase 1 metabolites; thus, it is not ideal for investigating the conjugated Phase 2 metabolites. This issue can be mitigated by utilising hydrolysis methods as necessary. A high-resolution LC-QToF-MS is used to detect and characterise the potential metabolites.

This study aims to investigate the metabolic pathways of several SCRAs belonging to the indazole carboxamide family, such as A-5F-PINACA and 3F-A-5F-PINACA, using HLM and the influence that different head group structures have on those pathways.

Methods: A standard solution of the SCRA is added to a buffered solution (pH 7.4) containing 0.01-0.03% acetonitrile and the NADPH regeneration system solutions. This is then pre-incubated for 5 minutes at 37 OC before metabolism starts. Human liver microsome (sourced from GenTest supplied by Corning Life Sciences/Discovery Life Sciences) is then added to the sample. It is incubated for 1-3 hours to allow the metabolism of the SCRA to be done. Control samples for the study include SCRA without HLM and HLM without SCRA. Ice-cold acetonitrile is then added to quench the reaction to prevent further metabolism. It is then centrifuged to precipitate out the denatured enzymes before passing it through a 0.22 µm filter and 5 µL injected for LC-QToF-MS analysis.

Analysis was performed on an Agilent 6510 high-resolution LC-QToF-MS with a Poroshell 120 EC-C18 column attached. The column utilised water and acetonitrile as mobile phases (0.1% formic acid added to both) at a 0.3 mL/min flow rate in gradient elution. Ionisation was done in positive mode with scanning ranges of 50-700 m/z (MS) & 50-700 m/z (MS/MS). Collision energies were set to 10, 20 and 40 eV, and data acquisition was done in untargeted mode. The data was then processed using MassHunter Qualitative software.

Results & Discussion: The data indicates that the SCRAs mainly underwent oxidation reactions forming mono-, di- and tri-hydroxylation and some N-dealkylation reactions. This was determined by using ms1 data to characterise the mass of the metabolite, which was structurally confirmed by the ms2 spectra generated from the metabolite breaking down under different collision energies. It was seen that the head groups influence the metabolism of the SCRAs, mainly in how the head group metabolises, making it harder to differentiate the metabolic profiles if they are similar.

All of the metabolism routes identified indicate that only Phase 1 metabolites were formed during the metabolism of the SCRA. However, this is expected as HLM is not well suited to produce the Phase 2 conjugation metabolites as it lacks the transferase enzymes responsible for producing them. This may be considered a limitation of a HLM method, but the Phase 2 metabolites can be converted to the Phase 1 metabolites using a hydrolysis process (either enzyme or alkaline) to address this issue. These metabolites may be used as biomarkers to indicate the presence or use of a SCRA within a human system.

Conclusion: This study successfully elucidated the metabolic pathways of several SCRAs with significant overlap in the structures while investigating the influence of the head group on the resulting metabolites. The in vitro study has provided good predictions for the metabolites, which were characterised by high-resolution LC-QToF-MS, which would be expected to be observed in an in vivo model. These metabolites may indicate the use of a specific SCRA that can be differentiated from the others in this study.

Analysis of over 250 novel synthetic opioids and xylazine by LC–MS-MS in blood and urine

NPS F-P-03

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Background & Aims: Overdose deaths due to synthetic opioids in the USA have increased by over 1400% from 2014 to 2022. Although these deaths are primarily caused by fentanyl, novel synthetic opioids (NSO) have also contributed to this increase, although the extent may be underestimated due to exclusion from testing scopes.

The goal of this qualitative method was to rectify this shortfall by including over 250 NSO for detection in blood and urine in a rapid and efficient LC-MS/MS method.

Methods: The extraction was accomplished using a simple protein precipitation of 150 µL of matrix, followed by size-exclusion particle filtration, followed by evaporation and reconstitution. Analytes were separated with an F5 column, and data acquired with a 12-minute MRM method. Data processing was automated and expedited using a custom-built query.

The scope encompasses 261 analytes including fentanyl analogs (fentalogs), nitazenes, utopioids, and other synthetic opioids. Since 59 of these analytes are non-separable isomers, they were grouped, resulting in 202 target analytes or analyte groups.

The method was validated in accordance with ASB/ANSI Standard 036. Applicability was assessed using 27 proficiency test samples and reanalysis of all 2022 authentic accidental overdose cases.

Results & Discussion: Out of the 642 total accidental overdoses in 2022, 617 were available for reevaluation, with 421 cases positive for NSO, fentanyl and fentalog precursors and metabolites, utopiods, nitazenes, xylazine, and opium compounds. Out of these, over 10% were previously undetected, indicating a new trend in San Francisco: 45 cases positive for fluorofentanyl, and 15 positive for xylazine. The ability to detect these analytes has enabled the cause and manner of death to be reassessed, resulting in more accurate findings and information for harm mitigation efforts by public health policymakers.

The rise in NSO use necessitates the improvement of detection capabilities, however, the high potency of NSO as well as the numerous isomers presents this as a challenge requiring both high sensitivity and high selectivity. The attempt to avoid detection and circumnavigate illegality have also led to the production of more variety of NSO over time, requiring an extensive scope. Previously published methods for NSO detection usually have scopes of less than 40 analytes, while this method targets 261 analytes and encompasses multiple NSO classes, including nitazenes, utopioids, and fentanyl and fentalog precursors and metabolites. Further, due to its increased prevalence, xylazine was also added and complimented contemporaneous NSO analysis.

Conclusion: To our best knowledge, this method is the most comprehensive NSO method published. Detection limits were at or below those found in previous literature. The increased scope has allowed for more accurate case findings and revealed trends in the community that were previously unknown, thus allowing stakeholders to develop better-targeted policies in response to the opioid overdose problem.

NPS F-P-04

Deaths involving novel benzodiazepines in Victoria, Australia

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Background & Aims: Novel benzodiazepines (NBz) are a class of New Psychoactive Substance (NPS) with increasing prevalence in recent years. Currently, 35 different NBz are monitored by the EMCDDA through the EU NPS Early Warning System at June 2023. Most of these NBz have largely unknown pharmacological and toxicological profiles; although many have high potencies increasing the risk of adverse health outcomes, with many linked to fatalities. Additionally, NBz are increasingly supplied in counterfeit medicines and may contain little to none of the advertised drug, further contributing to risk of harm.

In this presentation, deaths reported to the Victorian Coroners court where a NBz was detected were reviewed to determine the extent of their prevalence and possible contribution to the deaths as well as trends in their changing availability in the community.

Methods: NBZ positive cases were identified by interrogation of the Victorian Institute of Forensic Medicine (VIFM) case management system for the 5-year period between from 2018 to 2022. Data obtained included relevant context of the case (circumstances), toxicology report and medico-legal death investigation (pathology) reports, age, sex and the reported cause of death. All cases were subject to a full toxicological screen that included a comprehensive LC-MS/MS screen.

Results & Discussion: In total 133 cases contained at least one NBz with nearly half (65 cases) containing more than one NBz. Overall, 11 different NBz were detected, including etizolam (n=82), flualprazolam (n=43), clonazolam or 8-aminoclonazolam (n=30), bromazolam (n=15), clobromazolam (n=13), phenazepam (n=13), flubromazolam (n=12), flubromazepam (n=8), desalkylflurazepam (n=6), diclazepam (n=2), and estazolam (n=1). Some cases contained up to 7 different NBz. Concentrations of NBz varied, with the high potency NBz clonazolam found at lower concentrations (0.3 – 3.4 ng/mL, median 1.0 ng/mL) compared to less potent NBz such as etizolam (0.05 – 288 ng/mL, median 13 ng/mL).

Detections were transient over the 5-year period with early cases comprising of mostly etizolam, while the latest were more likely to contain bromazolam, clonazolam, clobromazolam, flubromazepam and phenazepam. In at least 27 cases, the deceased was described taking 'alprazolam' or 'Xanax' but alprazolam was not detected in 21 of these cases. Of the 133 deaths, 95 were considered drug-related, with at least one additional CNS depressant also present capable of contributing to death, with or without contribution of natural disease. However, no deaths were attributed to the toxicity of NBz alone. Of the 95 drug-related deaths, 75 were male with one-third aged less than 40 years. Other CNS depressant drugs commonly detected along with NBz were additional benzodiazepines legally available in Australia (97 cases), while the heroin metabolite 6-acetylmorphine was found in 23 cases, as well as methadone (20 cases), oxycodone (13 cases) and alcohol (32 cases).

Conclusion: This presentation demonstrates the changing diversity of NBz supplied in the Australian community and the importance of maintaining up-to-date analytical methods to ensure detection of new compounds distributed in the drug supply network. While there were no deaths related to intoxications with a NBz alone, their use with other CNS depressants has contributed to many deaths in recent years. This study highlights the risks to users associated with counterfeit or unapproved medications, particularly in combination with other drugs that produce CNS depressant effects.

HHC and derivatives – method development for analysis of semi-synthetic cannabinoids and their metabolites and its application to routine samples

NPS F-P-05

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Background & Aims: Semi-synthetic cannabinoids (SSC) refer to a group of compounds that can be produced from natural cannabinoids using simple chemical processes. In particular, hexahydrocannabinol (HHC) has spread rapidly in a large number of countries. Due to growing efforts to ban HHC, derivatives are also increasingly appearing. Additionally, Δ8-THC, known as the precursor of HHC, occurs repeatedly - typically in combination with Δ9-THC or in smaller amounts as a synthesis impurity of HHC.

The objective was to develop a comprehensive method for the simultaneous detection of HHC-diastereomers as well as Δ9-THC and Δ8-THC including their corresponding hydroxy- and carboxy-metabolites. This analytical framework was continuously extended by additional SSCs found in seized material to be able to qualitatively detect these - and whenever possible also their metabolites - in blood samples. The method is constantly applied to routine samples to gather prevalence data.

Methods: Chromatography was performed using a 1290 Infinity II LC system, coupled via Jet Stream interface (ESI) to a 6495C triple quadrupole mass spectrometer from Agilent. Various columns (including a C18-, a phenyl-hexyl-, a biphenyl, a fluorophenyl- and a chiral column) were tested in combination with different mobile phases.

The blood samples were pre-selected using THC routine-workflows: Samples with a positive immunochemistry result for THC or a positive urine-testing for THC by the police were qualitatively screened for the presence of HHC within the confirmatory chromatographic analysis for Δ9-THC. In case of a positive result for HHC, the samples were re-measured and quantified using the developed method. So far, 94 samples from a period between 10/2023-02/2024 were tested positive for HHC. In addition, six samples that could not be quantified using the routine-method due to the interference with Δ8-THC were re-measured. The collective is continuously updated with data from further cases.

53 SSC-containing seizures were obtained from the Rhineland-Palatinate State Criminal Police Office in a period 09/2023-02/2024, including cannabis flowers, hashish, vape liquids and edibles. For identification of SSC-derivatives beyond HHC, samples were analysed using different modes of mass spectrometry (initial MS2 scan followed by a product ion scan).

Results & Discussion: The C18- and the fluorophenyl-column with a step gradient and methanol as the organic phase turn out to be the best analytical starting points for method development. The separation of hydroxy-metabolites, including (R)- and (S)-HHC-OH, but especially Δ8-THC-OH and Δ9-THC-OH, was particularly challenging. Here, the fluorophenyl-column showed superior selectivity. Isotopic interferences (e. g. high THC-concentrations resp. its metabolites interfere with HHC and its metabolites) were another pitfall in method validation.

(R)-HHC-concentrations (LLOQ 0.5 ng/mL) in serum samples showed a median of 1.8 ng/mL (range 0.51-38 ng/mL, n=94). The (S)-diastereomer was detected in consistently lower concentrations in about half of the samples, with a median of 1.1 ng/mL (range 0.51-9.2 ng/mL, n=52). The (R)/(S)-ratios had a median of 3.4 (range 1.5-9.0). The median concentrations of 11-hydroxy-metabolites (LLOQ 0.5 ng/mL) were 0.95 ng/mL (range 0.51-5.0 ng/mL, n=54)

for (R)-OH-HHC and 0.78 ng/mL (range 0.55-1.3 ng/mL, n=13) for (S)-OH-HHC, those of 11-carboxy-metabolite (LLOQ 2.5) were 41 ng/mL (range 5.0-611 ng/mL, n=94) for (R)-COOH-HHC and 3.0 ng/mL (range 2.7-3.4, n=21) for (S)-COOH-HHC. The median (R)/(S)-ratio of the HHC-hydroxy-metabolites was 3.3 (range 1.5-5.5), which is identical to that of the parent compound, while the ratio of the carboxy-metabolites, at a median of 18 (range 1.9-114), is greatly increased. In about half of the samples HHC and Δ9-THC (median 2.4 ng/mL, range 0.5-62 ng/mL, n=52, LLOQ 0.5 ng/mL) were detected in parallel. Δ9-THC-COOH was even detected in 66 % of the samples (median 13 ng/mL, range 2.5-351 ng/mL, LLOQ 2.5 ng/mL). Δ8-THC-COOH was only present in 26 % of the samples in low concentrations (≤ 7.2 ng/mL) suggesting the uptake of HHC-containing products with low amounts of Δ8-THC as synthesis impurity. In the 6 samples with detection of Δ8-THC (range 0.6-4.7), Δ9-THC (range 2.6-8.8 ng/mL) was consistently found in higher concentrations.

Other derivatives besides HHC, including H4CBD, HHCP, HHCP-Acetate, Δ8-THCP, Δ8-THCP-Acetate, Δ9-THCP, Δ9-THCP-Acetate, HHC-Acetate and CBN-Acetate, were detected in similar percentages in seizures (26 %) and blood samples (21 %). Evidence for hydroxy- and carboxy-metabolites of THCP and HHCP was found in blood samples suggesting an analogous metabolism to their pentyl-homologs. In addition, metabolic deacetylation of acetates was proven in blood samples.

Conclusion: The method enables the detection of both diastereomers of HHC, Δ9-THC and Δ8-THC including their metabolites. To the best of the authors' knowledge, there is currently no published method that can reliably separate the hydroxy-metabolites of Δ8-THC and Δ9-THC.

SSC-derivatives as well as some of their metabolites could be detected at least qualitatively, because not every reference substance is commercially available so far. The parallel analysis of seizures and blood samples is a good way of reacting quickly to newly emerging SSC-derivatives and adapting the analysis accordingly.

NPS F-P-06

Oral fluid insights: Unveiling trends in illicit substance consumption and new psychoactive substances at Brazilian festivals through oral fluid analysis

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Background & Aims: The new psychoactive substances (NPS) encompass a diverse range of chemical compounds designed to produce psychoactive effects like traditional illicit drugs. Monitoring NPS involves a multifaceted approach encompassing surveillance, data collection, analysis, and dissemination of information to stakeholders at various levels. By aggregating and analyzing data from diverse sources, public health authorities can identify emerging threats, assess the prevalence and impact of NPS, and adjust interventions accordingly. One source of information is the monitoring of psychoactive substances at electronic parties and festivals. Drug use at parties is a phenomenon that has been prevalent in various social settings for decades. Parties, gatherings characterized by music, dancing, and socializing, often provide an environment where individuals may be inclined to experiment with substances. One of the primary reasons people use drugs in this context is to enhance their overall experience. However, it is essential to recognize that drug use at parties comes with inherent risks. Mixing substances, consuming unknown or adulterated drugs, and excessive use can lead to a range of adverse effects. One major concern regarding NPS is the lack of research on their long-term effects on human health. Many of these substances have not been tested in humans, and their potential risks are largely unknown. Additionally, due to the clandestine nature of their production, the quality and composition of these substances can vary significantly, further increasing risks for users. The objective of this study was to identify the consumption of illicit substances, including NPS in Brazil through the analysis of oral fluid (OF) samples collected at parties and electronic music festivals during 2023.

Methods: The OF samples were collected using the Quantisal device, at 8 different events from September 2023 to December 2023. Volunteers were ≥18 years old, received information about the research objectives, completed an anonymous and confidential questionnaire, and sample collection was performed according to the manufacturer's instructions. Six questions documented the age, gender, sexual orientation, education, estimated time of last consumption of any illicit psychoactive substance, and estimated time of last consumption of illicit psychoactive substances in the last 24 h. The samples were subjected to liquid-liquid extraction with MTBE and were analyzed by liquid chromatography tandem mass spectrometry (LCMS 8060, Shimadzu, Kyoto, Japan).

Results & Discussion: At 8 different events, 996 oral fluid samples were collected. Among the participants, 62.3% were male, and 59.0% reported being heterosexual. The mean age was 21 (ranging from 18 to 43 y.o., with a median of 23 y.o.), and approximately 26% were graduate students. Most (70.2%) reported consuming an illicit psychoactive

substance in the last three months (not considering marijuana). The presence of different psychoactive substances was observed in 63% of the samples, where two different psychoactive substances was observed in 42.4%, followed by three (30.8%), while others contained up to nine psychoactive substances. The main psychoactive substance detected in the oral fluid samples was MDA (72.2%), followed by MDMA (41.1%), Δ9-tetrahydrocannabinol (THC) (39.1%), cocaine (14.7%), and ketamine (11.1%). Additionally, methamphetamine, amphetamine, MDEA, lysergic acid diethylamide (LSD), N, N-dimethyltryptamine (DMT), and psilocin were also detected. Classic psychedelics, such as DMT and psilocin, were more prevalent at electronic music festivals than at parties. Only 0.4% of participants reported consuming a NPS, and among all the samples, 17% were positive for at least one these drugs. Among the NPS-positive samples, dipentylone (bk-DMBDP), methylone, pentylone (or dibutylone), deschloroketamine (DCK), 2-fluorodeschloroketamine (2-FDCK), 25B-NBOH, 25E-NBOH, ADB-BUTINACA, MDMB-4en-PINACA, and ADB-4en-PINACA were detected. In 75.5% of samples positive for synthetic cathinones, another stimulant substance such as MDA, MDMA, or methamphetamine was also detected. Dipentylone was the only synthetic cathinone that could be detected in isolation. In samples positive for ketamine derivatives, such as DCK or 2-FDCK, ketamine was present in only 6.3%. ADB-BUTINACA was the prevalent synthetic cannabinoid among the samples. In 34.3% of samples positive for some synthetic cannabinoid, THC was also detected. ADB-4en-PINACA was always detected concurrently with MDMB-4en-PINACA.

Conclusion: In this study we present data on consumption of illicit substances, including NPS, in social events in Brazil, using oral fluid investigation. MDA, MDMA and THC were the most prevalent traditional illicit drugs detected in the samples. Among the NPS-positive samples, dipentylone (bk-DMBDP) was the most prevalent. The data indicate a minimal correlation between the reported use of NPS and the detection of such substances. The ingestion of multiple illicit psychoactive substances was prevalent (63%), which can lead to serious adverse effects.

Protonitazepyne and metonitazepyne pharmacological profiling: Assessment of μ-, κ-, and δ-opioid receptors activation with a novel high-throughput non-radioactive GTP Gi binding assay

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Background & Aims: 2-Benzylbenzimidazole opioids, also known as nitazenes, represent a novel class of synthetic opioids that emerged on the illicit drug market in recent years. Despite legislative efforts that reduced the availability of some opioids like fentanyls, nitazenes appear to fill this gap by evading current legal controls. However, the risks associated with the consumption of these substances remain largely unknown, leaving users unaware of the potential dangers they pose.

Nitazenes exert their effects mainly through agonism at the opioid receptors in the central nervous system. They exhibit significantly greater potency than many other opioid drugs, thereby increasing the risk of life-threatening poisoning. The increasing consumption of these new synthetic opioids has indeed coincided with a surge in reports of overdose deaths attributed to nitazenes by the European Monitoring Centre for Drugs and Drug Addiction (EMCCDA). Consequently, a prompt and comprehensive investigation to elucidate their pharmacological action and associated risks is essential.

To address these concerns, this study aimed at characterizing the pharmacological profile of two recently marketed nitazenes, protonitazepyne and metonitazepyne. Their interactions with μ-, κ-, and δ-opioid receptors (MOR, KOR, and DOR, respectively) were assessed using a novel approach with a GTP Gi binding assay based on homogeneous time-resolved fluorescence (HTRF) detection.

Methods: Activation of G protein-coupled receptors (GPCRs) induces conformational changes in Gα proteins, leading to the exchange of GDP for a non-hydrolysable GTP analog in the Gα subunit binding pocket. HTRF technology combines fluorescence resonance energy transfer (FRET) technology with time-resolved measurement of fluorescence. In this assay, energy transfer occurs between a long-lived fluorescence donor (Europium cryptate, Eu3+ cryptate) and a short-lived fluorescence acceptor (d2-labeled anti-Gαi monoclonal antibody) when labeled biomolecules are in close proximity. After agonist-induced activation of GPCRs, energy transfer between Eu3+ cryptate and d2 generates a FRET signal proportional to the Gαi activation state, enabling pharmacological characterization of compounds. The protocol was performed in 96-well plates. Potency and efficacy of protonitazepyne and metonitazepyne were assessed after correction for non-specific binding. Concentrations ranged from 10-5 M to10-11M and were tested in duplicates; the experiments were performed in triplicates. Assay readouts were measured using a multilabel reader, capturing HTRF emissions at two wavelengths: 640 nm (donor) and 665 nm (acceptor). All values were normalized to the maximum

signal of each receptor reference compound arbitrarily set to 100% (MOR: fentanyl, KOR: U-50488, DOR: SNC-80). Concentration-response curves were fitted using GraphPad Prism to determine potency (EC50) and efficacy (Emax).

Results & Discussion: EC50 and Emax values for fentanyl, U-50488, and SNC-80, were consistent with published literature. Protonitazepyne showed a higher activity at MOR (EC50, ~3 nM; Emax, 160%) than metonitazepyne (EC50, ~33 nM; Emax, 105%). Protonitazepyne had a lower activation at KOR (EC50, ~2037 nM; Emax, 23%) compared to metonitazepyne with an EC50 and Emax of ~180 nM and 10%, respectively. As expected, low potencies and efficacies were measured at DOR for the two compounds: EC50 were ~981 nM and ~628 nM for protonitazepyne and metonitazepyne, and Emax were 59% and 45%, respectively.

Protonitazepyne and metonitazepyne showed distinct activation profiles at MOR, KOR, and DOR.

Protonitazepyne resulted around 10 times more potent combined with higher efficacy compared to fentanyl, while metonitazepyne potency was similar to fentanyl, with a slightly higher efficacy.

The KOR activation profile for protonitazepyne suggests potency around 50 times lower than that of U-50488 and low efficacy, while metonitazepyne potency was about four times lower than U-50488 and even lower efficacy. DOR activation profiles for protonitazepyne and metonitazepyne resulted in relatively low potency and efficacy when compared to SNC-80.

Conclusion: Considering the differences in activation at the three opioid receptors, this study may contribute to a better understanding of the potential risks and health implications of these compounds. The pronounced MOR activation exhibited by these compounds underlines their potential health risks due to severe adverse effects such as respiratory depression.

Even though in vitro results cannot directly be translated to in vivo effects, the HTRF GTP Gi binding assay offers an easy, rapid, safe, and cost-effective method for assessing opioid receptor activation of newly emerged drugs and can readily be expanded to other GPCRs.

NPS F-P-08

Deschloroketamine derivatives: Studies on their in vivo and in vitro metabolism and their microbial biotransformation in wastewater by means of hyphenated mass spectrometry

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Background & Aims: Recently, abuse was reported for the ketamine derivatives deschloroketamine (2-oxo-PCMe) and deschloro-N-ethyl-ketamine (2-oxo-PCE), which are expected to show dissociative and psychoactive properties. However, data on their biotransformation as well as on the biotransformation of further derivatives such as deschloro-N-cyclopropyl-ketamine (2-oxo-PCcP), deschloro-N-isopropyl-ketamine (2-oxo-PCiP), and deschloro-N-propyl-ketamine (2-oxo-PCPr) are scarce. Such data are needed e.g., to define urinary screening targets or allow their monitoring by wastewater (WW) based epidemiology. WW based epidemiology is an approach to estimate the consumption of drugs, drugs of abuse, and new psychoactive substances in a subpopulation, enabling short- and long-term monitoring. Hence, aims of the presented work were to study the in vivo and in vitro metabolism and detectability within standard urine screening approaches (SUSA) for five deschloroketamine derivatives. Furthermore, microbial biotransformation in WW of deschloroketamine derivatives and their in vivo metabolites in WW should be assessed.

Methods: All compounds (2-oxo-PCMe, 2-oxo-PCE, 2-oxo-PCcP, 2-oxo-PCiP, and 2-oxo-PCPr) were orally administered by gastric intubation to male Wistar rats in doses of 2 mg/kg body weight and urine and feces was collected over a period of 24 h. Urine and feces samples were further used for metabolite identification (i) and microbial biotransformation studies in WW (ii). In vitro samples were generated by incubating deschloroketamine derivatives (25 µM, final concentration) using pooled human liver microsomes (pHLM, 1 mg microsomal protein/mL) and were used for metabolite confirmation (i). Control samples without enzymes and blank incubations without deschloroketamines were prepared and all incubations were performed in dublicate.

(i) Sample preparation was performed by urine precipitation, solid-phase extraction (SPE), glucuronidase and arylsulfatase cleavage in combination with both liquid-liquid extraction (LLE) and SPE. In vitro samples were terminated by addition of 50 µL of ice-cold acetonitrile, centrifuged at 18,407 x g for 2 min, and supernatants were then used for analysis. Samples were analyzed via liquid chromatography-high resolution tandem mass spectrometry (LC-HRMS/MS). Additionally, in vivo samples were analyzed using gas chromatography coupled to mass spectrometry (GC-MS) after partial urine hydrolysis followed by LLE and acetylation. Detectability of deschloroketamine derivatives and their identified metabolites was tested in the in vivo samples by LC-HRMS/MS or GC-MS-based SUSA.

(ii) Biotransformation of deschloroketamine derivatives and their metabolites in WW was assessed by incubating the parent compounds at concentrations of 0.1 mg/L or the rat urine or rat feces samples in freshly collected, untreated, influent WW over a period of 24 h at 22 °C. Incubations of rat urines were performed after preconcentrating 1 mL of rat urine via precipitation using methanol (50:50, v/v), centrifugation, and supernatant evaporation to dryness under a stream of N2. Incubation was done after reconstitution in 4 mL of WW for 24 h. Feces samples (1 g) were suspended in WW and incubated for 24 h. Sample preparation was performed using Isolute HCX SPE cartridges (Biotage, Uppsala, Sweden) followed by analysis via LC-HRMS/MS.

For both, (i) and (ii), chromatographic separation was performed by a Thermo Fisher Scientific (TF) Dionex UltiMate 3000 LC (UHPLC) system and a TF Accucore Phenyl-Hexyl column (100 x 2.1 mm ID, 2.6 µm). Detection of analytes was done using a TF Q-Exactive, operated in full scan and data dependent acquisition (DDA). GC-MS analysis was performed using a Hewlett Packard (HP, Agilent Technologies, Waldbronn, Germany) 5890 II gas chromatograph combined with a HP 5972 MSD mass spectrometer.

Results & Discussion: In total, 39 metabolites were tentatively identified in vivo (29 phase I and 10 phase II), including N-dealkylation, hydroxylation, hydroxylation with further oxidation to a ketone or carboxylic acid, glucuronidation, and N-acetylation. In vitro pHLM incubations confirmed 19 of 29 phase I metabolites identified in vivo. The ketone, carboxylic acid and dihydroxylation metabolites were not detected after using pHLM. Using the GC-MS based SUSA only the N-dealkylation metabolite could be detected. In comparison, the LC-HRMS/MS-based SUSA allowed identification of specific metabolites for every deschloroketamine derivative, except for 2-oxo-PCiP. After WW incubations over 24 h, all parent compounds and their metabolites except O-glucuronidated and Nacetylated metabolites were detectable. This is in line with previous findings where phase II conjugates were identified to be unstable in WW.

Conclusion: All five deschloroketamine derivatives showed similar metabolic pathways after in vivo rat experiments, with most metabolites also confimed using in vitro pHLM experiments. Comparison of LC-HRMS/MS and GC-MS SUSA allowed detection of substance-specific metabolites in case of LC-HRMS/MS. Though the GC-MS-based SUSA only allowed for the identification of unspecific metabolites, it may at least indicate ingestion of a deschloroketamine derivative. Subsequent analysis using more sensitive approaches, e.g., SPE, might then be used for substance-specific identification. WW incubations demonstrated that the deschloroketamines and most of their metabolites are still detectable in WW influent and thus can be used as analytical targets in the context of WW based epidemiology.

Identification of hexahydrocannabiphorol metabolites in human urine

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NPS F-P-09

Background & Aims: Hexahydrocannabiphorol (HHCP) was the most widely spread semi-synthetic cannabinoid in Switzerland after the ban of hexahydrocannabinol (HHC) in March 2023. It has been used as legal substitute to Cannabis or HHC until the ban of "dibenzopyran cannabinoids" in October 2023. HHCP seems to be a far more potent and longer lasting cannabimimetic than HHC or THC, as suggested by in vitro binding affinities of similar compounds to the human CB1 receptor and anecdotal reports from drug forums.

Our aim was to identify urinary metabolites to prove the consumption of HHCP. To our knowledge no human metabolites of HHCP have been reported yet.

Methods: After ingestion of 4 mg HHCP dissolved in 2 mL olive oil (52 % 9R-HHCP and 21 % 9S-HHCP, GC-MS area percentage) by a volunteer, urine samples were collected and analyzed with LC-QqTOF for metabolite identification (phase I and phase II). A LC-QqLIT method was developed for the deglucuronidated metabolites. The samples were extracted with SPE (Chromabond C18, non-endcapped, Macherey-Nagel) using a validated method for "THC and metabolites", derivatized to their respective TMS-derivatives and analyzed with GC-MS (EI).

Results & Discussion: Hydroxyalated phase-I-metabolites and their respective glucuronides were found. Hydroxylation occured on the alicyclic part or on the side-chain of the molecule. In addition dihydroxylated metabolites were found, with one hydroxy-group on the side-chain and another hydroxy-group on the alicyclic moiety of the molecule.

LC-MS data of phase-I- and phase-II-metabolites and GC-EI-MS data for the trimethylsilylated phase-I-metabolites are presented. These metabolites are suggested for LC-MS and GC-MS (after silylation) for qualitative screening procedures.

Conclusion: Similar to the urinary metabolites of HHC hydroxylation of HHCP occurs on the alicyclic part or on the side-chain. In contrast to HHC a second hydroxylation can also be observed. We think that this is common for cannabinoids with a longer side chain to facilitate excretion.

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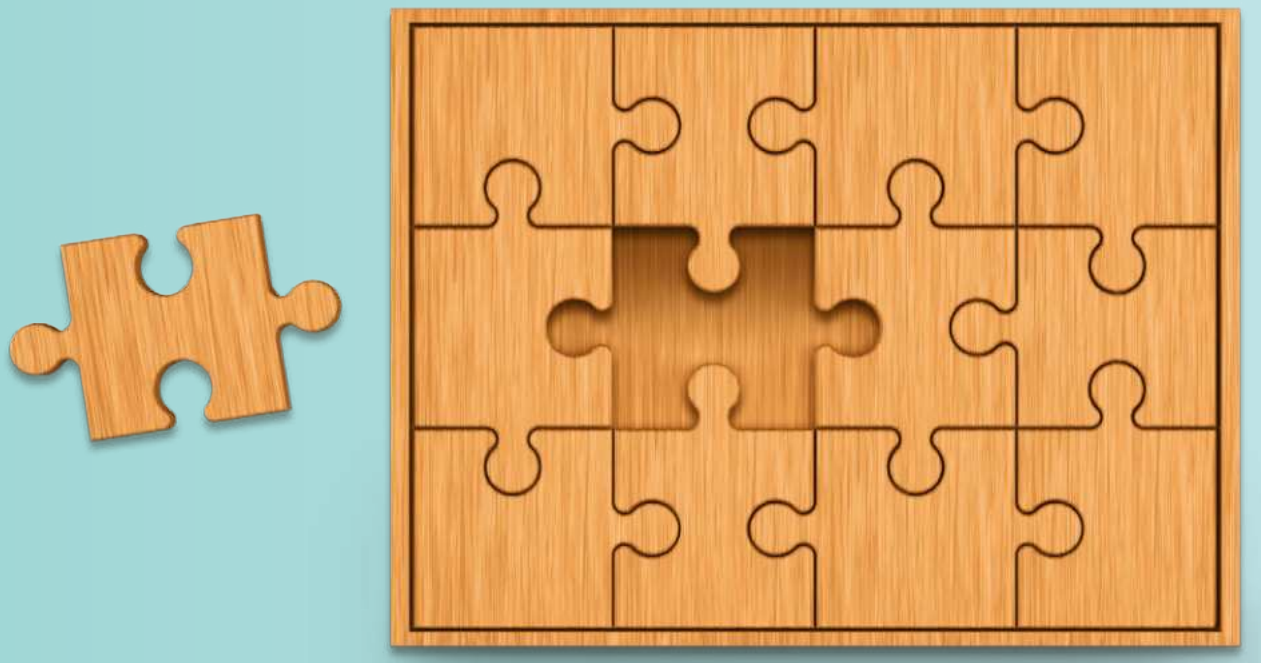

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A forensic investigation of urinary metabolites of ADB-BUTINACA and MDMB-4en-PINACA.

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Background & Aims: Indazole derivative synthetic cannabinoid receptor agonists (SCRAs) have become more prevalent in abuse in the last three years. From the data gathered from the seized items analyzed at the Bahrain Ministry of Interior (MOI) Forensic Science Lab (FSL), the most prominent SCRAs were ADB-BUTINACA and MDMB-4en-PINACA, however due to the unavailability of metabolic data and the absence of an enzyme immunoassay for these substances' metabolites, detection in authentic urine samples was not possible until metabolic profiling of these compounds was published in scientific papers. This study aims to use the published LC–QTOF fragmentation features of the drugs and their metabolites for their identification in authentic human urine of suspected SCRAs abusers.

Methods: A holistic screening for all the published proposed metabolites for these substances was done. Four authentic urine samples with suspect abuse of a variety of SCRAs were tested for confirmation of the method. The samples were extracted using SPE, Rapid Trace, 2 mL of urine sample were loaded onto an Oasis SPE cartridge (60 mg, 3 cc), which had been previously washed with methanol (2 mL) and deionized water (2 mL). The cartridge was then washed with water (2 mL) and subsequently eluted with methanol (2 mL). The eluent was evaporated to dryness under 30 °C nitrogen stream and reconstituted in 200 µL of a 50-50 mixture of formic acid and acetonitrile, and 15 µL was injected for LC-QTOF-MS analysis. Urine samples were not hydrolyzed for the analysis. Data acquisition was done on two levels, first using MS acquisition in positive ions [M+H] mode and screening using the Find by Formula function in the Agilent quantitative workflow software using the formulas reported in the papers. The matched protonated molecules were then targeted using Targeted MS/MS, and the fragmentation pattern was then compared with the published fragmentation of the molecule in the papers.

Results & Discussion: Table 1: Details of detected metabolites in authentic human urine

Proposed by	ID	Biotransformation	Formula	Exact mass [M+H]+ (m/z)	Mass Error (ppm)	Average RT (min)
Kronstrand et al.	m1	ADB-BUTINACA Amide hydrolysis + dehydrogenation	C18H23N3O3	330.1812	*-0.90859*	6.12
	m2	ADB-BUTINACA Amide hydrolysis	C18H25N3O3	332.1969	-0.602	7.76
Watanabe et al. and Yester et al.	m3	MDMB-4en-PINACA Ester hydrolysis	C19H25N3O3	344.1969	-3.2926	9.26
Gu. Et. Al.	m4	MDMB-4en-PINACA ester hydrolysis and N-butanoic acid	C18H23N3O5	362.171	-0.9327	6.87
Watanabe et al. and Yester et al.	m5	MDMB-4en-PINACA ester hydrolysis and dihydrodiol	C19H27N3O5	378.2023	0.7932	6.41

Gu. Et. Al.	m6	MDMB-4en-PINACA Ester hydrolysis, dehydrogenation, hydroxylation	C19H23N3O6	390.1659	-5.38233	6.36
	m7	MDMB-4en-PINACA N-glucuronidation with ester hydrolysis	C20H25N3O9	452.1663	-2.43273	5.93
	m8	MDMB-4en-PINACA Ester hydrolysis, glucuronidation	C21H27N3O9	466.182	-0.21451	6.14
	m9	MDMB-4en-PINACA N-dealkylation, glucuronidation	C25H33N3O9	520.2289	-1.73001	7.06
	m10	MDMB-4en-PINACA Dihydrodiol, hydroxylation, glucuronidation	C26H37N3O11	568.25	-1.23185	6.53

Table 2: Detection of SCRAs Metabolites in Authentic Urine Samples

ID	MS Area Case 1	MS Area Case 2	MS Area Case 3	MS Area Case 4
m1	2.58E+07	5.18E+07		
m2	8.22E+07	1.61E+09		
m3	7.19E+06		2.69E+07	3.33E+07
m4	2.71E+07			
m5	2.39E+08		3.56E+07	6.45E+06
m6		8.77E+06		
m7		1.22E+07		
m8		7.95E+06		
m9			2.45E+07	
m10			2.89E+06	

Conclusion: Using LC–QTOF-MS analysis for a comprehensive metabolic profile, proved effective in identifying SCRA intake across two substances. The ability to detect a wide range of metabolites enables forensic laboratories to keep pace with evolving drug trends and provides a valuable tool in the fight against drug abuse.

The analysis revealed the complex nature of SCRA abuse, with individuals often consuming mixtures of substances. This complexity underscores the necessity of a comprehensive screening approach, as shown by the targeted detection of various proposed metabolites.

It is notable to consider that hydrolysis of this sample could deconjugate other metabolites that could have been detected.

NPS P–11

Non-fatal overdose with 3-MeO-PCE: Identification of phase I and phase II metabolites in various biological fluids and metabolomic studies

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Background & Aims: We report a case of severe intoxication after snorting 3-methoxyeticyclidine (3-MeO-PCE), also known as methoxieticyclidine, a dissociative anaesthetic analogue of phencyclidine, sold online as a designer drug. A man in his 20s was found unresponsive in his home and taken to the emergency department at a local hos-

pital. He was comatose and severely respiratory depressed. Hetero anamnesis revealed that the patient may have consumed the substance MeO-PCE, which he had bought on the internet. Following supportive care, the patient fully recovered.

Methods: A white powder found at the patient's home was dissolved in methanol and analysed by GC-MS and UPLC-MS/MS. Blood, urine, head hair and pubic hair samples collected at the hospital were subjected to a comprehensive toxicological screening using both UPLC-MS/MS and LC-HRAM-Orbitrap-MS. Incubation of 3-MeO-PCE with human hepatic microsome S9 fractions was performed to study its metabolism.

Results & Discussion: 3-MeO-PCE was identified in the powder and detected in all biological samples. The concentrations of 3-MeO-PCE at hospital admission were 153 and 1369 ng/mL in blood and urine respectively. Cocaine and metabolites, cannabinoids and benzodiazepines were also found in blood and urine. Cocaine, ketamine, MDMA, THC, and fentanyl were detected in hair samples. A large number of 3-MeO-PCE phase I and phase II metabolites were identified by LC-HRAM-Orbitrap-MS in blood, urine, hair and in the hepatic microsome incubation medium.

Conclusion: Toxicological findings confirmed the use of 3-MeO-PCE in the intoxicated patient and also revealed a history of polydrug use. The use of LC-HRAM-Orbitrap-MS allowed an easy identification of the new psychoactive substance and its phase I and phase II metabolites in both biofluids and hepatic microsome incubation medium.

In vitro metabolite identification of ethyleneoxynitazene, a new 2-benzylbenzimidazole opioid

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Background & Aims: New synthetic opioid (NSO) availability on the drug market has increased since the mid-2000s. Recently, the 2-benzylbenzimidazole analogs, also called nitazenes, have dominated this subclass of novel psychoactive substances. Nitazenes are opioid receptor agonists inducing euphoria and strong analgesic and anesthetic effects, with a pharmacological potency comparable to that of fentanyl. They have been involved in numerous drug-related deaths and near-fatal overdoses in Europe and America, motivating their legal control in many countries. Detection of nitazenes is crucial for demonstrating substance use, but their concentrations in biological matrices are low due to their metabolic degradation and generally low active doses, and that makes their detection challenging. The identification of metabolite biomarkers is therefore essential to identify exposure to nitazenes. The aim of this study is to identify specific biomarkers of consumption of ethyleneoxynitazene, which was first reported in February 2023 to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA).

Methods: Ethyleneoxynitazene was incubated for 3 h with 10-donor-pooled human hepatocytes to simulate in vivo metabolism. After protein precipitation, incubates were analyzed by liquid chromatography-high-resolution tandem mass spectrometry (LC-HRMS/MS) with a separation on a biphenyl column and detection in full-scan MS and data-dependent MS/MS following electrospray ionization in positive and negative modes (Q Exactive from Thermo Scientific). Compound Discoverer (Thermo Scientific) mined the raw data to facilitate comparison between incubates and controls, online library comparison, and automatically search for a combination of metabolic reactions. Analyses were supported by in silico metabolite predictions in humans with GLORYx open-access software (Hamburg University, Germany).

Results & Discussion: GLORYx predictions for ethyleneoxynitazene produced 11 first-generation metabolites through N-deethylation, N-oxidation and hydroxylation at the N-ethyl chain, hydroxylation at the dihydrobenzofuran group, dihydrofuran opening to the corresponding ethanol/ethanal, and oxidative deamination to the corresponding ethanol. Predicted second-generation metabolites were combinations of the aforementioned reactions and phase II conjugations. Fifteen ethylenoxynitazene metabolites were identified in hepatocyte incubations. The main transformations were N-deethylation, hydroxylation, and dihydrofuran ring opening to the corresponding ethanoic acid. Additional transformations such as oxidation, oxidative deamination, desaturation, nitro reduction, O-Glucuronidation, N-acetylation, and combinations thereof, were identified. The most intense metabolite was N-deethyl ethyleneoxynitazene, which was approximately ten times more intense than the second most intense metabolite.

Conclusion: In preliminary pharmacological studies, ethyleneoxynitazene has shown low potency and efficacy compared to the most potent nitazenes. The drug, however, has already been identified in several countries since 2023 and is legally controlled in several countries such as Germany and the United Kingdom. Detecting ethyleneo-

xynitazene and biomarkers of exposure is therefore necessary in clinical and forensic casework. We assessed ethyleneoxynitazene metabolism in human hepatocyte incubations through in silico metabolite predictions, LC-HRMS/MS analysis, and software-assisted targeted/untargeted data mining. N-Dealkylation was a major transformation in ethyleneoxynitazene metabolism, as observed with the metabolism of other nitazene opioids such as isotonitazene, metonitazene, and etodesnitazene. We therefore suggest N-deethyl ethyleneoxynitazene as the main biomarker of exposure to ethyleneoxynitazene. Interestingly, several nitazene N-deethyl metabolites were shown to be active in opioid receptor activation experiments, suggesting that this might be true for ethyleneoxynitazene as well. Nitro reduction and further N-acetylation might be underestimated with the present in vitro model, although the analysis of authentic specimens is necessary to verify this assumption. These results will help clinical and forensic toxicologists to identify cases of ethyleneoxynitazene use, facilitating further verification of the presented findings.

NPS P-13

In vitro characterization of protonitazene metabolites produced by human liver microsomes and subsequent comparison with biological samples from two fatal intoxications

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Background & Aims: Protonitazene is a novel synthetic benzimidazole derivative with potent opioid effects, which has been sold over the internet as a designer drug since 2019. The nitazene class appeared in France in Spring 2023 and was involved in clusters of serious poisoning cases in Occitanie and in French Islands located in the India Ocean, including deaths. The authors report the characterization of several protonitazene metabolites, using in vitro incubation with human liver microsomes, and subsequent comparison with urines samples from two fatal protonitazene intoxications.

Methods: The formation of protonitazene metabolites was investigated by in vitro incubation, with human liver microsomes, using a Tris-HCl-MgCl2 solution and a cofactors mixture. The enzymatic reaction was performed at 37 °C for 120 min and stopped by the addition of methanol. Supernatants were injected in an ultra-performance liquid chromatography-quadrupole time of flight-mass spectrometry (UPLC-Q-TOF-MS). Subsequently, urine specimens were also analyzed by UPLC-Q-TOF-MS, after a hydrolysis step and a liquid-liquid extraction. For all analyses, the chromatographic separation was performed using a HSS C18 column and a 15 min gradient elution. Detection was performed using a high-resolution mass spectrometer (XEVO G2XS Q-TOF, Waters Corporation, Milford, MA, USA) operated in positive and sensitivity mode. In MS scanning, data were acquired from 50 to 1000 m/z and collision energy ranged from 10 to 40 eV. UNIFI was used for data, chromatograms, spectra acquisition and to predict potential metabolites.

Results & Discussion: In vitro human liver microsomes incubation was performed to investigate phase I metabolization pathways. The experience produces protonitazene and three metabolites under these conditions: protonitazene (C23H30N4O3: m/z [MH+] 411.2387); desethyl-protonitazene (C21H26N4O3: m/z [MH+] 383.2072); 5-amino-protonitazene (C23H32N4O: m/z [MH+] 381.2649) and 4-hydroxy-nitazene (C20H24N4O3: m/z [MH+] 369.1930). Base on its chemical structure, 4-hydroxy-nitazene seems to be a universal metabolite of the nitazene analogs. In the two urines specimens, protonitazene was confirmed (2.3 and 2.9 ng/mL, respectively). The three metabolites were identified, which enhances the evidence of protonitazene consumption. It was not possible to quantify the metabolites, since no reference standard is available. However, the calculated area ratios (metabolite/parent protonitazene), higher than 1, suggest that the identification of these metabolites can increase the time of detection in urine, in case of protonitazene consumption. 4-hydroxy-nitazene is the major metabolite, with a calculated ratio of 15.9 and 38.3, respectively. In addition, two others substances were observed both after in vitro incubation and in the urines, corresponding to [MHprotonitazene+]-73 and [MHprotonitazene+]-60, with calculated ratios also higher than 1. These possible metabolites are currently under investigation.

Conclusion: Protonitazene metabolism was studied in vitro using human liver microsomes and allows the identification of three metabolites: desethyl-protonitazene, 5-amino-protonitazene and 4-hydroxy-nitazene. For the first time, these metabolites were identified in human urine specimens, collected during autopsies of two abusers. One can anticipate that because metabolites were found with chromatographic responses higher than the response of the parent protonitazene, these metabolites will allow an extension of the window of detection. The 4-hydroxy-nitazene appears to be the major metabolite and may be the target substance in human urine.

Detection of nitazenes in vape juice

NPS P–14

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Background & Aims: The continuous emergence of novel synthetic opioids (NSO) on the recreational drug market is creating an additional challenge for drug tracking agencies and laboratories to meet. In recent years, a class of synthetic opioid originally developed in the 1950s as opioid analgesics called the nitazenes has been detected in the illicit drug supply and implicated in overdose mortality. Nitazenes vary greatly in potency and purity and thus often require only a small amount to cause acute intoxications. Their recent detection in illicit vape juice which resulted in accidental and fatal drug overdoses poses a major challenge for public health officials.

In this study, a collection of vape juice products was purchased from vaping stores and screened for a panel of 15 nitazenes. A quantitative LC-MS/MS method was developed to accurately detect low-levels of nitazenes in the vape juice products.

Methods: Three vape juice samples were purchased from vaping stores. The samples were diluted 1000 times in methanol and directly injected for LC-MS/MS analysis. These samples were used as the matrix to establish the calibration curve and were not intended for the identification of nitazene adulteration.

Liquid chromatography was performed using a Shimadzu LC-40 at a flowrate of 0.6 mL/min using a Phenomenex Kinetex 2.6 um F5 100 A 50x2.1 mm (Phenomenex Torrance, CA). The injected sample volume was 10 µL. Mobile phases A and B were 10 mM ammonium formate in Optima grade water Optima grade methanol with 0.02% formic acid, respectively. Samples were then injected into the QTRAP 4500 system where and MS/MS data were acquired using positive electrospray ionization. The scheduled MRM algorithm was used to collect the appropriate amount of data points for quantifiable data. A single acquisition method consisting of 34 MRM (30 for the analytes and 4 for the internal standards) was created and used for analysis of the samples.

Results & Discussion: The three vape juice products were merely used to provide a blank matrix for this method. To ensure the absence of nitazene in the samples, all three vape juice products were tested for the presence of nitazene compounds. All three products came back negative and were used as a blank matrix for the rest of the experiments.

The three vape juice products were diluted 1000 times in methanol and spiked with the 15 nitazenes. These spiked vape juice solutions were injected at six concentration levels ranging from 0.5 pg/mL to 100 ng/mL to assess the quantitative performance of the developed method. Excellent linearity was observed across the concentration ranges analyzed with R2 values greater than 0.99 for all the nitazenes targeted in this panel. The sample preparation procedure used in this experiment demonstrated excellent recoveries (%recoveries >80%). In addition, the method showed low variability between injections (% CV <10 for all the analytes included in the panel), demonstrating the high level of reproducibility of the method.

Conclusion: A comprehensive workflow for the detection of 15 nitazenes in vape juice was developed. A fast and simple preparation method combined with a robust acquisition method enabled accurate quantitation of the panel of 15 nitazenes compounds. The optimized LC conditions resulted in separation of the drugs in a 15.5-minute runtime while the use of the MRM workflow enabled sensitive and accurate quantification of the 15 nitazene compounds across six calibration levels. The fast and simple sample procedure for sample preparation demonstrated high recovery levels for the 15 nitazenes targeted in this workflow. In addition, the robustness of the developed workflow enabled reproducible and sensitive quantification of the analytes, making this method suitable for screening vape juice products for the presence of potent and highly toxic nitazene compounds.

The comparative metabolism study of 4-fluor-3-methyl-alpha-PVP and alpha-PCYP, two synthetic cathinone type stimulants using UHPLC-QToF

NPS P–15

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Background & Aims: 4-fluor-3-methyl-alpha-pyrrolidinopentiophenone (MFPVP) and alpha-pyrrolidinocyclohexylphenone (alpha-PCYP) are two cathinone type psychostimulants. Both substances were first reported to EMCDDA in Sweden. MFPVP appeared in 2020 and it was reported in Hungary in November 2020. Alpha-PCYP first appeared in 2013 and it was reported to EMCDDA in July 2016 in Hungary. These two psychostimulants were relatively popular on the European drug market. Until 2024, they were rarely confiscated in Hungary. However, this year the number of authentic urine samples screened and confirmed positive for these two compounds is growing in Hungary. Forensic toxicology aims to determine drug abuse by analysing biological samples. However, these samples might contain only metabolites of the consumed substance. Therefore, it is essential to study the metabolic pathways and metabolic profile of any newly emerging drug.

Methods: In the Department of Forensic Toxicology of HIFS, the metabolic profiling assays of MFPVP and alpha-PCYP were performed on a Waters Xevo G2-XS UPLC-QToF system in MSe mode. Waters_Connect software was applied. We analysed blank in vivo, blank in vitro, spiked in vitro (pHLM and pS9) samples and in vivo urine samples (n=3 and n=1 for MFPVP and alpha-PCYP, respectively) from users. Phase I and Phase II metabolites were investigated and ranked according to their relative abundance. Major metabolic pathways were determined.

Results & Discussion: In this metabolism study, seven in vivo urinary metabolites of MFPVP and seven urinary metabolites for alpha-PCYP were tentatively identified. For MFPVP, six Phase I metabolites via reduction of the keto moiety (MA01), oxidation (MA08), via the combination of these two (MA02, MA03), carboxylation (MA07), ring-opening followed by carboxylation (MA10) and one Phase II metabolite via the glucuronidation of the carboxylated metabolite (MA06) were detected in three authentic urine samples. For alpha-PCYP, six Phase I metabolites via reduction of the keto moiety (MB02), ring-opening followed by carboxylation (MB01), keto-reduction and oxidation (MB03), keto-reduction and hydroxylation (MB04, MB05) and via hydroxylation (MB06) and one Phase II metabolite via hydroxylation and glucuronidation (MB07) were detected in one urine sample. According to the metabolite ranking study, four major metabolites of MFPVP (MA02, MA03, MA06 and MA08) and three major metabolites of alpha-PCYP (MB01, MB02 and MB03) were more abundant than the parent compound. MA01, MA07, MA10, MB054, MB05, MB06 and MB07 were also dominant. MA04, MA05 and MA09 were detected only in vitro.

Conclusion: The major metabolic pathways of MFPVP and alpha-PCYC were determined. These pathways were proved to be similar. The main metabolic biotransformations are reduction of the keto moiety, hydroxylation of the benzyl ring, oxidation of the cyclohexyl ring or the pentyl chain and the opening of the pyrrolidine ring followed by carboxylation. This metabolic profile is highly similar to the metabolism of analogous compounds (alpha-PVP, alpha-PiHP). The difference in the relative abundance of the metabolites is due to the different side groups. The metabolite ranking study presented that MFPVP and alpha-PCYP are both intensively metabolised in humans. Consequently, monitoring of tentatively identified dominant metabolites might facilitate the sensitive and selective detection of the consumption of these cathinones.

NPS P-16

In silico prediction and forensic detection of cinnamylpiperazines biomarkers of exposure

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Background & Aims: Opioid-related drug overdoses have been rising for the past thirty years in the U.S. Synthetic opioid-related deaths accounted for over 82% of opioid deaths in 2020. Many illicitly manufactured synthetic opioids show higher potency than morphine or heroin. AP-237 and its derivatives (2-methyl-AP-237, para-methyl-AP-237, and AP-238) have gained popularity due to their online easy availability. In 2019, 2-methyl-AP-237 and AP-238 appeared on the illicit market and were identified through drug seizures. AP-237 is considered a weak opioid receptor agonist. However, pre-clinical studies have shown that 2-methyl-AP-237 and AP-238 are also m-receptors agonists presenting the same or higher potency than morphine. Few data are available for the prevalence and potency of para-methyl-AP-237, but it was already observed in forensic cases. Forensic and clinical identification of these substances is challenging and usually relies on the identification of their metabolites and the parent drug in biological samples. The study of the metabolism of NPSs is extremely important for the proper forensic identification of these compounds in case of intoxication. This study aimed to identify the metabolites of four cinnamylpiperazines using in silico predictions and experimental data using Liquid Chromatography – Quadrupole Time-of-Flight – Mass Spectrometry (LC-QTOF-MS).

Methods: In silico prediction of the metabolites was applied by combining four different software. Way2Drug was used to predict the sites of metabolism (SOM), SwissADME and ADMETlab for pharmacokinetic information, and GLORYx to predict phase I and phase II metabolites for all 4 drugs. Different concentrations of the target compounds were incubated with microsomes from different species (human, rat, mouse, and rabbit), NADPH, NADPH regeneration system, and buffer at 37°C at different times with a maximum time of 90 minutes. After incubation, cold acetonitrile was added to stop the reaction. A centrifugation step was conducted, and the supernatant was collected for LC-QTOF-MS analysis sing two systems, an Exion Liquid Chromatograph coupled to an SCIEX X500B QToF and a Shimadzu Nexera 40 Liquid Chromatograph coupled to an SCIEX X500R QToF.

Results & Discussion: A total of 34, 43, 30, and 35 phase I and phase II metabolites were predicted for AP-237, 2-methyl-AP-237, AP-238, and para-methyl-AP-237, respectively. The in-silico predictions suggest that the cinnamylpiperazines share the same SOM, a reactive carbon adjacent to the nitrogen-substituted ring, for most metabolism reactions catalyzed by the CYP3A4, CYP2D6, CYP2C19, CYP2C9, and CYP1A2 isoforms. The predicted pharmacokinetic data showed that the compounds analyzed are inhibitors of CYP2D6 and CYP2C19, and only AP-238 doesn't inhibit CYP2C19. All drugs are substrates of CYP1A2, CYP2C9, CYP3A4, but AP-238 is also substrate for

CYP2C19. Experimental results showed that the main metabolites for all the compounds are the result of hydroxylation, reduction, desaturation, and oxidation, with important differences among species, showing the predominance of hydroxylation in rabbits, when compared to humans. In silico predictions were shown to be effective as an initial tool for the detection of NPS metabolites and for the comparative assessment of interspecies metabolism. From the metabolites predicted 67%, 72%, 56%, and 51% for bucinnazine, 2-methyl-AP-237, para-methyl-AP-237, and AP-238, respectively, were found experimentally.

Conclusion: The in-silico approach allowed the identification of the most reactive carbon in all compounds studied suggesting that the main site for metabolization is the carbon adjacent to the nitrogen-substituted ring due to its electronegativity and structural availability. In addition, metabolization differences were found among the species. The understanding of species differences in drug metabolism improves our abilities to predict the pharmacological and toxicological properties of a given compound in man from experimental data obtained in animals. Overall, this dual approach for the metabolites' identification enabled to propose relevant candidates as future biomarkers of consumption for the cinnamylpiperazines analogues currently in the market. The addition of these biomarkers to mass spectrometry libraries will help diagnose intoxication cases.

Exploring the metabolic pathways of the newly emerged synthetic cannabinoid receptor agonists ADMB- and Cumyl-3TMS-PrINACA in human urine specimens

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Background & Aims: The rapid proliferation of new psychoactive substances (NPS), particularly synthetic cannabinoid receptor agonists (SCRAs), poses significant challenges to drug testing laboratories. Routine detection methods, especially for urine samples, struggle to keep pace with the evolving landscape due to the swift metabolization of parent compounds. In late 2022, peak shifts were observed in a routine LC-MS/MS method for isolated SCRA detection in urine, attributed to a N-butyl-OH metabolite of ADB-BINACA (other name: ADB-BUTINACA, ADMB-BINACA, N-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-butyl-1H-indazole-3-carboxamide) and a metabolite hydroxylated at the N-butyl side chain of Cumyl-4CN-BINACA (other name: Cumyl-4CN-BUTINACA, 1-(4-Cyanobutyl)-N-(2-phenylpropan-2-yl)-1H-indazole-3-carboxamide). They were later identified as primary metabolites associated with the newly emerged SCRAs ADMB- and Cumyl-3TMS-PrINACA. This study aimed to elucidate the human in vivo phase I metabolism of these novel compounds. Both share a common scaffold with a unique trimethylsilyl (TMS) group as a side chain moiety, differing only in the linked group. In vitro assays using pooled human liver microsomes (pHLMs) were employed to confirm and plausibly identify metabolites, supported by liquid chromatography-quadrupole-time-of-flight-mass spectrometry (LC-QToF-MS) and in silico metabolite prediction using GLORYx and BioTransformer 3.0.

Methods: ADMB- and Cumyl-3TMS-PrINACA were incubated separately in pHLM assays at a parent compound concentration of 10 µg/mL in triplicate for 30 minutes to identify potential metabolites. A total of 22 human urine samples found positive for ADMB- and/or Cumyl-3TMS-PrINACA were enzymatically hydrolyzed, followed by protein precipitation and liquid-liquid extraction. LC-HESI-QToF-MS analysis in positive mode was conducted in fullMS and bbCID mode, supplemented by autoMS/MS for clear MS/MS spectra.

Results & Discussion: LC-QToF-MS analysis revealed main metabolites of ADMB-3TMS-PrINACA and Cumyl-3TMS-PrINACA with cleavage of the 3TMS-group and subsequent carboxylation at the N-propyl scaffold, isobaric to an N-butyl-OH metabolite of ADB-BINACA and Cumyl-4CN-BINACA. The presences of the respective N-propionic acid explained the shifted signals observed for the transitions for the monohydroxylated metabolites monitored. As the primary analytical targets for both SCRAs the N-propionic metabolites as well as the N-propyl-OH metabolites are suggested. Additionally, substance-specific biomarkers still carrying the TMS moiety such as mono- and dihydroxylations were identified.

Conclusion: Detecting synthetic cannabinoid intake in urine specimens remains a significant challenge for NPS testing laboratories. LC-HRMS/MS facilitated phase I metabolite detection of ADMB-3TMS-PrINACA and Cumyl-3TMS-PrINACA in human urine, corroborated by in vitro pHLM assays. The highlighted phase I metabolites may serve as reliable urinary targets for detecting ADMB- and Cumyl-3TMS-PrINACA use.

NPS P–18

Cumyl-CBMEGACLONE, Cumyl-NBMEGACLONE and Cumyl-NBMINACA: Human phase I metabolism of structurally related synthetic cannabinoids bearing a cumyl moiety and a cyclobutyl methyl or norbornyl methyl tail

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Background & Aims: Synthetic cannabinoid receptor agonists (SCRAs) continue to show high prevalence on the new psychoactive substances (NPS) drug market, with several structural modifications implemented since their first detection. Around 2019-2020 new SCRAs bearing a cumyl moiety emerged: Cumyl-CBMEGACLONE, carrying a cyclobutyl methyl (CBM) tail, and Cumyl-NBMEGACLONE, characterized by a norbornyl methyl moiety (NBM) attached to the γ-carbolinone core. The NBM tail substructure is also carried by Cumyl-NBMINACA, the indazole carboxamide analog of Cumyl-NBMEGACLONE.

This study aimed at evaluating the human phase-I metabolism of Cumyl-CBMEGACLONE, Cumyl-NBMEGACLONE and Cumyl-NBMINACA to provide suitable urinary markers to prove their consumption.

Methods: For identification of the in vivo phase-I main metabolites of Cumyl-CBMEGACLONE, Cumyl-NBMEGACLONE and Cumyl-NBMINACA, a total of 14 authentic urine samples obtained from different SCRAs users were analyzed, after enzymatic cleavage of glucuronides, by liquid chromatography time-of-flight mass spectrometry (LC-qToF-MS). In vitro phase I metabolites were generated by applying a pooled human liver microsomes (pHLM) assay and were used as plausibility controls. Results were compared with metabolites of structurally similar SCRAs.

Results & Discussion: Fifteen human phase I metabolites of Cumyl-CBMEGACLONE were identified in vivo. On the basis of the abundance in authentic samples and comparison with metabolites of structurally related SCRAs, the products of monohydroxylation at the CBM group and at the core are suggested as urinary biomarkers for detecting and monitoring the consumption of Cumyl-CBMEGACLONE.

For Cumyl-NBMEGACLONE, 9 human phase I metabolites were detected, among which M8 and M9, both products of monohydroxylation at the norbonyl methyl tail, are recommended.

Thirteen metabolites of Cumyl-NBMINACA were detected and, despite the limited number of urine samples, M7 and M3, built by di- and tri-hydroxylation, are suggested as specific biomarkers to monitor Cumyl-NBMINACA consumption.

Conclusion: In the present study, the human phase I metabolism of three SCRAs bearing a cumyl moiety and a CBM or NBM tail is reported, based on LC-qToF-MS data of authentic urine specimens, confirmed by analysis of in vitro pHLM assays. The pHLM assay proved to be a valuable tool for metabolite prediction, although the analysis of authentic samples remains inevitable to identify biomarkers that could be used as specific and unequivocal proof of consumption.

NPS P–19

New Substance Psychoactive apprehensions in São Paulo State, Brazil (2022-2023)

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Background & Aims: The growing emergence of new psychoactive substances (NPS) has been a challenge for the institutions responsible for substance control and toxicologists, as new substances are constantly incorporated into the market, and marketed in ways that make it difficult for them to be identified during inspection. NPS can be considered of high risk to public health, since much is still unknown regarding toxicity, effects, damage and dissemination.

In Brazil, there is a challenging scenario, due to the territorial dimension, structural diversity of these substances and the difficulty in acquiring reference standards. In addition, the majority of the apprehensions are carried out

by the State Police, and as there is no centralization of apprehension data for each State, there is no clear profile of the real Brazilian situation in relation to NPS. Seeking to understand the dynamics behind the NPS that continue to appear in apprehensions around the world, including Brazil, this report brings together data on apprehensions from the Narcotics Examinations Center (NEE) of the Technical-Scientific Police Superintendence (SPTC), from July 2022 to December 2023.

Methods: Gas chromatography coupled to mass spectrometry (GC-MS), Fourier-transform infrared (FTIR) spectroscopy, High-resolution mass spectrometry (HRMS) and Nuclear magnetic resonance (NMR) spectroscopy were employed in accordance with the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) guidelines to identify of substances.

Results & Discussion: Over the period studied, July 2022 to December 2023, 87 different substances were identified, isolated or in the form of a mixture. According to the data, synthetic cannabinoids are the class with the greatest diversity of substances, representing 45.4% of the substances detected, followed by synthetic stimulants (18.6%) and hallucinogens and dissociatives (17.5%).

Considering the number of apprehensions, in the last six months of 2022, a total of 905 cases of apprehensions containing NPS were recorded. In the first six months of 2023, 2,245 cases were registered; and in the last six months of the same year, 1,786 cases were registered. On all occasions, synthetic cannabinoids constitute the most prominent class, further reinforcing the importance and concern for this class.

Analyzing the ten cities with the biggest apprehensions, the total number of apprehensions does not exceed 183 cases, with the exception of São Paulo city. The synthetic cannabinoids prevail in São Vicente, Carapicuíba, Guarulhos, Franco da Rocha, Osasco and Praia Grande cities. On the other hand, Santos and Cajamar cities have a greater number of apprehensions of synthetic stimulants. Meanwhile, in the city of São Paulo, with 2,892 apprehensions of NPS, synthetic cannabinoids and stimulants represent 51.9% and 35.6%, respectively. The NEE-SPTC analyses all samples apprehended in the city of São Paulo, while other cities have other centers for apprehension analysis. Therefore, the data presented is not capable of representing all apprehensions from adjacent cities.

Analyzing the half-yearly panorama, there is a significant increase in apprehensions that occurred between the second half of 2022 and the first months of 2023, in which 131 NPS apprehensions from December became 595 in March 2023. As 2023 progresses, apprehensions decrease, but still remain high compared to the second half of 2022. Finally, the apprehensions were mainly of samples containing isolated substances. However, the presence of mixtures stays high. Since, in both the second half of 2022 and 2023, synthetic cannabinoids were mostly apprehended in the form of mixtures, which can mean unknown risks to those who use it and makes the work of health professionals more difficult.

Conclusion: In conclusion, according to the data, synthetic cannabinoids are the most important class in the State of São Paulo, followed by synthetic stimulants and hallucinogens and dissociatives. The majority of the drugs apprehended are in isolated form and the city of São Paulo is the one that stands out the most.

Finally, this report provides important information regarding the dissemination of NPS in the State, that can assist in the development of public policies, influence future measurements of the NEE-SPTC and provides a basis for the development of future academic projects focused on Forensic Chemistry and Toxicology.

Hexahydrocannabinol (HHC) intoxication resulting in acute agitation and respiratory depression. Detection of HHC phase I and phase II metabolites in blood and urine samples by LC-HRAM-Orbitrap-MS

NPS P-20

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Background & Aims: Hexahydrocannabinol (HHC) is a chemically stable cannabinoid synthesized from cannabidiol (CBD) via either the intermediates Δ9-tetrahydrocannabinol (Δ9-THC) or Δ8-tetrahydrocannabinol (Δ8-THC). In the period 2021-2023 HHC appeared on the USA, UK, and EU drug markets, and was commonly found as (9R) and (9S) epimers. Typical HHC-containing products are vape pens, e-liquids, oils, tinctures. Several EU countries, including Italy, and Switzerland did not regulate the sale or use of HHC until 2023.

A male subject, 54 years old, bought a vial (labelled as "HHC Amnesia Terminator 20%") of an oily product from a cannabis shop and ingested about 5 mL. Two hours later he developed agitation, nausea, and vomiting. On admis-

sion to the emergency department, he presented with anxiety, respiratory depression, and tachycardia. On-site urine toxicology screening returned positive for cannabinoids. Whole blood and urine samples were collected from the patient on admission, and whole blood approximately twelve hours later, for further toxicological analysis. The patient was discharged 19 hours from hospital admission.

Here we describe the detection of both HHC epimers in the oily product purchased and consumed by the patient by gas chromatography – mass spectrometry (GC-MS) and liquid chromatography - high-resolution accurate-mass Orbitrap mass spectrometry (LC-HRAM-Orbitrap-MS), as well as the detection of HHC epimers and their phase I and phase II metabolites in the patient's whole blood and urine samples by LC-HRAM-Orbitrap-MS.

Methods: Following mixing with organic solvents and dilution the oily product was analysed with GC-MS [Thermo Scientific Trace GC Ultra/Polaris Q MS system; EI full scan (m/z 50–400); 30 m Agilent HP-5MS UI analytical column; oven temperature 180-300 °C (20 °C/min)] and LC-HRAM-Orbitrap-MS [Thermo Scientific Ultimate 3000 UHPLC system/Q-Exactive Focus MS instrument; Hypersil Gold C18 (50 x 2.1 mm, 1.9 mcm) analytical column at 30 °C; full scan (m/z 70-1000) and all-ion fragmentation (normal collision energies 17.5, 35.0, 52.5 eV) positive-ion acquisition modes at mass resolution of 70,000]. (9R)-HHC and (9S)-HHC certified analytical standards were used for comparative analysis.

Whole blood and urine samples (200 mcL) were subjected to protein precipitation with 700 mcl of cold acetonitrile:methanol 2:1 (v/v) after adding the internal standard (IS) THC-D9. In addition, urine samples underwent differential enzymatic (b-glucuronidasi/Arysulfatasi, 60 °C, 2 h) and alkaline (NaOH 10 M, 60 °C, 2 h) hydrolysis before protein precipitation. LC-HRAM-Orbitrap-MS analysis was carried out in full scan conditions, as described before, and in data-dependent acquisition (dd-MS2) confirmation mode (resolution 17,500, isolation window 3.0 m/z, isolation offset 1.0 m/z, normal collision energies 17.5, 35.0, 52.5 eV), according to an inclusion list of exact mass values comprising HHC and expected phase I and phase II metabolites, therefore isolating and fragmenting MH+ ionic species at m/z 317.2475 (HHC), 333.2424 (hydroxy-HHCs), 347.2217 (carboxy-HHCs), 493.2796 (HHC-glucuronides), 509.2745 (hydroxy-HHC-glucuronides), and 523.2538 (carboxy-HHC-glucuronides).

Results & Discussion: GC-MS and LC-HRAM-Orbitrap-MS analyses confirmed the presence in the oily product of (9R)-HHC and (9S)-HHC, in a 2:1 ratio. A small amount of the synthetic intermediate Δ8-THC was also found.

(9R)-HHC and (9S)-HHC epimers were both detected in blood. They were not detected in plain urine, but only after alkaline hydrolysis, and even more so after enzymatic hydrolysis, as expected. Several phase I and phase II metabolites were detected in both blood and urine samples. Metabolites were hydroxy-HHCs, carboxy-HHCs, HHC-glucuronides, hydroxy-HHC-glucuronides, and carboxy-HHC-glucuronides. Identification was based on evaluation of their chromatographic behaviour compared to the parent compounds, accurate mass measurements of their MH+ ions in full scan conditions, evaluation of their MH+ isotopic patterns, accurate mass measurements of MH+ collision-induced product ions. HHC-glucuronides, hydroxy-HHC-glucuronides, and carboxy-HHC-glucuronides were identified due to characteristic fragmentation patterns matching those of the parent drugs or phase I metabolites from which they derived. Urine alkaline hydrolysis increased the abundances of carboxy-HHCs, whereas enzymatic hydrolysis increased the abundances of hydroxy-HHCs. These results helped in the identification of metabolites.

Conclusion: Some recent publications described the detection of HHC and some metabolites in different biological matrices. In one of those studies, no separation of HHC epimers or identification of glucuronides was achieved. In another, HHC phase I and phase II metabolites were detected, but in urine only. Therefore, the discrimination of HHC epimers and the detection of their phase I and phase II metabolites in both whole blood and urine samples following an acute human intoxication represent a step forward from previous studies.

NPS P-21

Intoxication with a new piperidino-nitazene, etonitazepipne: Metabolism and postmortem blood concentration

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Background & Aims: Following the strengthening of laws controlling fentanyl analogs in the United States and China in 2019, new opioid receptor agonist subclasses emerged on the novel psychoactive substance (NPS) market to circumvent legislation. In particular, 2-benzylbenzimidazole opioids, also called nitazenes, have become popular over the last years. Some of these nitazenes, such as etonitazene, have an estimated analgesic potency a thousand times higher than that of morphine, and have been involved in many acute intoxication cases and fatalities worldwide. Considering their potency and therefore their low expected concentration in biological specimens, the detection of nitazene

metabolites often is the only means to prove exposure in forensic and clinical toxicology. Etonitazepipne is a nitazene with a pharmacological potency similar to that of fentanyl that was first identified in Germany in January 2022. We hereby report the first case of fatal intoxication involving this substance in Germany, in February 2022. Etonitazepipne was quantified in the individual's femoral blood taken at the autopsy, and metabolites were identified in human liver microsome (HLM) incubations and the individual's urine to identify specific biomarkers of consumption.

Methods: Etonitazepipne was quantified in blood with the standard addition method after liquid-liquid extraction. Analysis was performed by liquid chromatography (LC) (Nexera-X2 LC-30AD, Shimadzu) with a pentafluorophenylpropyl column, and tandem mass spectrometry (MS/MS) (QTRAP-5500, Sciex) in multiple reaction monitoring (MRM) mode after positive electrospray ionization. To study etonitazepipne phase I metabolism, the substance was incubated with pooled HLM for 2 h in phosphate buffer at 0.5 mol/L and an NADPH regenerating system. After liquid-liquid extraction, incubates were analyzed by LC (UltiMate 3000, Dionex) with a biphenyl column, and high-resolution MS/MS (HRMS/MS) in full-scan MS/data-dependent MS/MS mode after positive and negative electrospray ionization; an inclusion list composed of metabolites predicted in silico with GLORYx software (Hamburg University, Germany) was compiled to prioritize fragmentation. Data screening was performed with Compound Discoverer (Thermo Scientific) with a targeted and a non-targeted workflow. The individual's urine taken at the autopsy was analyzed under the same conditions with and without β-glucuronidase hydrolysis.

Results & Discussion: Etonitazepipne blood concentration was 8.3 ng/mL. Intra- and inter-day repeatability was 5.6 and 4.9%, respectively, with an analytical recovery of 85% and a matrix effect of +30%. Limits of detection and quantification were 0.1 and 0.5 ng/ml, respectively. A total of 19 metabolites were found in HLM incubates, among which 11 were also detected in the urine specimen; 4 additional metabolites, among these 3 phase II metabolites, were identified in urine. The main metabolic transformations were hydroxylation and oxidation at the piperidine ring, opening of the piperidine ring to the corresponding N-pentanoic acid, and O-dealkylation of the ethoxy group. Other minor reactions included hydroxylation and oxidation at the benzimidazole core or the ethoxyphenyl group, nitro reduction, and O-glucuronidation.

Conclusion: Considering the lack of data on etonitazepipne toxic and fatal blood concentrations and its in vitro potency similar to that of fentanyl, the cause of death was attributed to etonitazepipne toxicity (Toxicological Significant Score of 3, according to Elliott e al. 2018). After glucuronide hydrolysis, the main urinary biomarkers of the opioid were the metabolites produced through piperidine ring opening to N-pentanoic acid (which was not reported in a previous etonitazepipne-positive case), O-deethylation, and combination of hydroxylation and O-deethylation, the LC-HRMS signal of the parent drug being at least five times lower than that of the three proposed metabolite biomarkers in the present case. Considering μ-opioid receptor activation experiments conducted on structural analogs, O-deethyl metabolites might be active and therefore contribute to etonitazepipne toxicity.

In vitro and *in vivo* metabolic study of three new psychoactive β-keto-arylcyclohexylamines

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Background & Aims: Since the 2000s, an increasing number of new psychoactive substances have appeared on the illicit drug market. β-keto-arylcyclohexylamine compounds play important pharmacological roles in anesthesia; however, because these new psychoactive substances have been rapidly increasing illicit recreational use, the lack of detailed toxicity data are of particular concern. Therefore, analysis of their metabolites can help forensic personnel provide references and suggestions on whether a suspect has taken an illicit new psychoactive β-keto-arylcyclohexylamine. The present study investigated the in vitro and in vivo metabolism of three β-keto-arylcyclohexylamines.

Methods: In vitro and in vivo models were established using zebrafish and human liver microsomes for analysis of phase I and II metabolites of deschloro-N-ethyl-ketamine, fluoro-N-ethyl-ketamine, and bromoketamine by liquid chromatography–high resolution mass spectrometry.

Results & Discussion: Altogether, 51 metabolites were identified. Most of the phase I metabolites produced by arylcyclohexylamines were formed through permutations and combinations of N-dealkylation, hydroxylation, reduction, or dehydration reactions, and on the basis of which phase II metabolites were produced by glucuronidation and sulfation at the hydroxyl or amine positions.

Conclusion: Hydroxy-deschloro-N-ethyl-ketamine, hydroxy-fluoro-N-ethyl-ketamine, and hydroxy-bromoketamine were recommended as potential biomarkers for documenting intake of deschloro-N-ethyl-ketamine, fluoro-N-ethyl-ketamine and bromoketamine respectively, in clinical and forensic cases.

NPS P-23

Analytical method development and metabolite identification of 2-Fluoro-2-oxo PCE: Implications for concurrent ketamine abuse

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Background & Aims: 2-Fluoro-2-oxo PCE (2-(ethylamino)-2-(2-fluorophenyl)-cyclohexanone, 2-fluoro-N-ethylnordschloroketamine, 2-F-2-oxo PCE) emerged in Australia in 2022, exhibiting dissociative anesthesia akin to ketamine. In Korea, 2-F-2-oxo PCE emerged in the form of white powder in May 2023 and was detected in approximately 1.9% of all seized materials (6,076 samples) during 2023, compared to a ketamine detection rate of 5.0%. This emergence is prompting concern due to its circulation in the illicit drug market alongside ketamine. Lack of knowledge regarding its metabolites accentuates the need for research. This study aimed to develop a method for simultaneous analysis of ketamine and 2-F-2-oxo PCE in urine using LC–MS/MS and identify metabolites using LC–QTOF/MS.

Methods: To determine whether ketamine abusers were actually using 2-F-2-oxo PCE, 0.1 mL of urine confirmed to contain ketamine was extracted using solid-phase extraction and then analyzed by LC-MS/MS. Subsequently, to confirm the presence of metabolites associated with 2-F-2-oxo PCE, urine samples underwent methanol protein precipitation and were analyzed by LC-QTOF/MS. Additionally, urine extracted using a SPE method was subjected to analysis by GC/MS.

Results & Discussion: In the LC–MS/MS method, the Limits of Detection (LOD) and Limits of Quantification (LOQ) for 2-F-2-oxo PCE and ketamine were determined to be 0.2 and 0.5 ng/mL, respectively. LC–MS/MS analysis demonstrated satisfactory precision and accuracy. Among the urine samples collected by the Seoul Institute of National Forensic Service from May to October 2023, 74 samples (53 males and 21 females) containing ketamine were analyzed by LC-MS/MS to confirm the presence of 2-F-2oxo PCE. The mean urine concentration of 2-F-2-oxo PCE was 1631 ng/mL (ranging from 1.1 to 23655, n=25), whereas the mean ketamine concentration was 1362 ng/mL (ranging from 1.5 to 49996, n=74). Metabolites including deethylated and dehydrogenated forms of 2-F-2-oxo PCE were identified by LC-QTOF/MS, with GC/MS providing complementary data.

Conclusion: The emergence of 2-F-2-oxo PCE in Korea as a new psychoactive substance (NPS) alongside ketamine highlights the evolving landscape of illicit drug markets. The analytical methods employed in this study, including LC-MS/MS, LC-QTOF/MS, and GC/MS, demonstrate their effectiveness in detecting and characterizing 2-F-2-oxo PCE and its metabolites in biological samples. Identification of metabolites associated with 2-F-2-oxo PCE provides valuable insights for proactive urine analysis and contributes to understanding its pharmacokinetics and toxicological profile. The findings emphasize the importance of ongoing surveillance and research efforts to address the challenges posed by emerging synthetic drugs and mitigate associated public health risks.

NPS P-24

UHPLC-MS/MS methodology for analysis of new synthetic opioids and hallucinogens in whole blood

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Background & Aims: New Psychoactive Substances (NPS) are a real contemporaneous threat, due to their potency, dangerousness, and lack of control/monitoring. The NPS group that has grown the most is the synthetic opioids group, where fentanyl and its analogues stand out. However, the emerging concerning synthetic opioids are nitazenes. Due to their high potency, even minimal consumption doses can lead to severe health effects or even fatal overdoses, making them a public health issue. Notwithstanding, it is also important to remain vigilant towards more "traditional" psychoactive substances like hallucinogens, as they have been associated with both intentional and unintentional poisonings/intoxications. This is particularly relevant now that they are also being used for clinical purposes. Therefore, it is essential to establish analytical methodologies for monitoring these compounds. As a result, the aim of this study was to develop, optimize and validate an easy to use, fast, simple, sensitive, robust, and routine method for the analysis of new synthetic opioids (fentanyls and nitazenes) and hallucinogens in whole blood.

Methods: The present work describes a method for screening, qualitative confirmation, and quantification of fentanyl, norfentanyl, sufentanil, carfentanil, isotonitazene, metonitazene, LSD, 2-oxo-3-hydroxy-LSD, mescaline, and psilocin. The sample preparation step consisted in 50 µL of whole blood protein precipitation with refrigerated acetonitrile containing formic acid and was optimized using Full Factorial Design of Experiments, to achieve the best conditions for compounds extraction from matrix. Following centrifugation, the resulting supernatant extract was directly injected into an ultra-high-performance liquid chromatograph coupled to a triple quadrupole linear ion trap mass spectrometer (Sciex UHPLC-QTRAP-MS® 6500+) and analyzed in a 5-minute run in multiple reaction monitoring mode with 2 transitions for each compound. The developed analytical methodology was fully validated according to the guiding principles of the ANSI/ASB Standard 036. To confirm its applicability in a real context, the proposed methodology was applied to the analysis of authentic forensic samples.

Results & Discussion: Regarding validation parameters, the methodology linearity was verified between 1 and 20 ng/mL. The precision and accuracy were satisfactory, with values <20% and within ±20%, respectively. The limits of detection were between 0.1 and 1 ng/mL, depending on the compound. Dilution ratios were also successfully evaluated. Selectivity was confirmed by analyzing spiked samples containing several therapeutic drugs and other drugs of abuse.

Conclusion: The proposed methodology provides a valuable and powerful tool for toxicology laboratories, enabling the simultaneous identification, confirmation, and quantification of two families of psychoactive substances, namely synthetic opioids and hallucinogens. Its speed, simplicity, effectiveness, and reliability make it particularly advantageous for routine implementation.

Keywords: New Synthetic Opioids; Hallucinogens; Whole Blood; Toxicology; Protein Precipitation; UHPLC-MS/MS

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Emergence of the new synthetic cathinone 3,4-EtPV: Successfully circumventing the German legislation on new psychoactive substances

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Background & Aims: The fast-paced emergence and rapid proliferation of novel psychoactive substances (NPS) with varying chemical structures pose significant challenges globally, prompting regulatory and analytical responses. In 2016, Germany implemented a generic legislative approach – the Act on New Psychoactive Substances (NpSG) – to control the distribution of NPS, including synthetic cathinones. The consumption of NPS has been linked to detrimental health effects ranging from acute intoxication to long-term physical and psychological harm. Synthetic cathinones, a class of stimulant substances structurally related to cathinone found in the khat plant, have gained popularity as recreational drugs due to their stimulant properties.

A prior study by Pulver et al. highlighted an unsuccessful attempt to bypass the German regulations through the online sale of 3,4-Pr-PipVP, falsely labeled as "3,4-EtPV". This study aims to investigate another sample obtained from a recent online purchase, also labeled as "3,4-EtPV" (1-(bicyclo[4.2.0]octa-1,3,5-trien3-yl)-2-(pyrrolidin-1-yl)pentan-1-one), as a potential new NPS to exploit loopholes in the current version of the German NpSG. To facilitate the identification of additional biomarkers for urine analysis with a potentially extended detection window compared to the parent substance, pooled human liver microsome (pHLM) assays were conducted.

Methods: Identification was performed using gas chromatography-mass spectrometry (GC-EI-MS), liquid chromatography ion trap mass spectrometry (LC-MSn), and quadrupole time-of-flight mass spectrometry (LC-QToF/MS) as well as nuclear magnetic resonance (NMR) spectroscopy. Additionally, pHLM incubations were analyzed by LC-QToF/MS to tentatively identify and obtain reference spectra of in vitro phase I metabolites.

Results & Discussion: NMR spectroscopy confirmed the 3,4-EtPV structure, complemented by LC-MSn insights into fragmentation pathways. HR-MS analysis revealed the distinctive mass fragments m/z 187.1121 (C13H15O+, delta -2.1 ppm) possibly resulting from a loss of the pyrrolidine ring, as well as the respective fragments probably formed by alpha-cleavage at the carbonyl carbon m/z 131.0491 (C9H7O+, delta <0.1 ppm) and m/z 126.1277 (C8H16N+, delta <0.1 ppm). A McLafferty rearrangement of the m/z 187.1121 fragment to m/z 145.0647 (C10H9O+, delta 0.7 ppm) and a subsequent Wagner-Meerwein-type rearrangement followed by the loss of CO could be a possible pathway to

the fragment m/z 117.0698 (C9H9+, delta 0.9 ppm). Among several metabolites, the main phase I biotransformation products generated from the pHLM assays were identified as benzocyclobutene core hydroxylation and pyrrolidine ring hydroxylation metabolites. These metabolites may serve as potential urinary biomarkers until 3,4-EtPV positive authentic human specimens are available, facilitating broader insights into its in vivo metabolism. Notably, since the benzocyclobutene core is not covered by the NpSG, 3,4-EtPV currently is a legal drug in Germany.

Conclusion: This study highlights regulatory challenges associated to the dynamic NPS landscapes and emphasizes the need for proactive measures. The emergence of 3,4-EtPV reveals deliberate exploitation of legal loopholes in the German NPS legislation. Analysis of online-sourced samples aids in understanding the evolving drug market, necessitating continuous monitoring. Moreover, the investigation has uncovered that samples from unknown sources labeled similarly may vary in their primary compound over time, posing additional health risks. This instance emphasizes the need for drug checking services and/or careful drug monitoring to support harm reduction efforts in response to the emergence of unknown novel psychoactive substances.

NPS P-26

First identification of the new benzimidazole opioid "F-Etonitazene" in a liquid drug preparation

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Background & Aims: New synthetic opioids (NSO) are analgesic substances which continue to appear on the drug market, posing a life-threatening risk of respiratory depression due to overdose. Many of these drugs are highly potent and carry the additional risk of accidental exposure. Since 2019, 2-benzyl benzimidazole type opioids (`nitazenes') have become increasingly important, first and foremost isotonitazene. In Germany, nitazenes almost completely replaced fentanyl analogs with respect to NSOs newly appearing on the drug market. In the EU, 15 nitazenes have been detected so far, with almost all of them now under intensive monitoring due to increasing numbers of reports of severe and fatal intoxications. This work aimed to identify and analytically characterize a novel NSO suspected in a test-purchase of a yellow-colored, viscous liquid.

Methods: After alkaline diethyl ether extraction, the novel NSO was analytically characterized employing gas chromatography-mass spectrometry (GC-EI-MS) as well as liquid chromatography quadrupole time-of-flight (HR-MS/MS) mass spectrometry. The final structural confirmation was performed using nuclear magnetic resonance (NMR) analysis.

Results & Discussion: GC-EI-MS analysis of the alkaline diethyl ether extract revealed a single peak in the total ion chromatogram with a mass spectrum similar to etonitazene. The congruent base peak at m/z 86, corresponding to the iminium ion formed after alpha-cleavage of the diethylamine moiety in etonitazene, suggests that this feature is also present in the unknown NSO. The molecular ion was detected at m/z 414 instead of m/z 396, indicating an 18 Da mass shift, which could be explained by the exchange of a hydrogen by a fluorine atom. Other fragments congruent with the EI mass spectrum of etonitazene were found at m/z 107 and 58, referring to the oxybenzyl and the amine moiety, respectively. The fragment at m/z 153 distinguishes the novel NSO as it also contains the mass shift of 18 Da compared with the fragment observed for etonitazene at m/z 135, that refers to the ethoxybenzyl moiety. HR-MS/MS analysis confirmed the molecular composition of the NSO to be C22H27FN4O3 and revealed additional characteristic fragment ions at m/z 342.1250 (C18H17FN3O3+, < 0.1 ppm), 296.1321 (C18H17FN2O+·, 0.5 ppm), and 153.0711 (C9H10FO+, 0.5 ppm) further corroborating the location of the fluorine at the terminal position of the ethoxy group. The final confirmation of the structure proposed on the basis of mass spectrometric fragmentation was achieved using NMR analysis.

Conclusion: We describe the first identification and analytical characterization of the latest benzimidazole opioid, F-Etonitazene, in illicit drug products. F-Etonitazene was available in the form of a viscous liquid and not as a pure solid, reducing the risk of accidental exposure by dusts. At the time of purchase, F-Etonitazene was not covered by the German NpSG and thus will create the need to update the respective group definition.

NPS P-27

Synthetic cannabinoid receptor agonist (SCRA) acute toxicity and drug seeking in adult zebrafish

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Background & Aims: Belonging to the category New Psychoactive Substances (NPS), the synthetic cannabinoid receptor agonists (SCRAs) are a class of compounds that binds to cannabinoid receptors (CB1 and CB2) and mimics the effects of delta-9-tetrahydrocannabinol (Δ9-THC). Many SCRAs are potent CB1 agonists, leading to more intense THC-like symptoms such as increased heart rate, vomiting, agitation, confusion, and hallucinations. This class of NPS is described as a high potency group and can even lead to death. An important parameter regarding the recreational use of NPS is the assessment of the possibility of causing dependence in those who consume them. The use of zebrafish for toxicological assays has been increasing for many applications in the last few years and this model presents several advantages over other in vivo approaches. Regarding responses to drugs of abuse, zebrafish have also demonstrated complex processes such as sensitization, tolerance, withdrawal, drug seeking, extinction, and relapse. Considering the importance of understanding parameters related to SCRA intoxications, the objective of this project is to determine the acute toxicity of SCRAs and evaluate seeking behavior in adult zebrafish after exposure, in comparison to opioid self-administration.

Methods: All zebrafish experiments were approved by the University of Utah IACUC. To determine the toxicity of SCRAs in embryonic development stages, the Fish Embryo Toxicity (FET) test was performed, consisting of early exposure of the embryos to the substances. Exposures occurred in embryos between 0- and 4-days post-fertilization (dpf), at doses of 0.001-10 µM. Hence, at least one endpoint was recorded as indicator of lethality and other changes as indicators of non-lethal effects. On the other hand, for larval experiments, the Maximum Tolerated Concentration (MTC) test was performed, with larvae between five and eight dpf, at the same doses tested in FET. For its evaluation, signs of acute locomotor impairment, deformations, lack of heartbeat, and death were registered. Finally, a well-established protocol previously developed in the Peterson laboratory will be used to evaluate seeking behavior in adult zebrafish using a test arena consisting of a plastic tray with enclosed submersible square platforms connected to a larger water reservoir equipped with a pump and illuminated with a warm white light source. A Raspberry Pi minicomputer and two infrared cameras are connected to the system, one for each of the two platforms used, "active" (yellow) and "inactive" (white). When the fish cross the "active" platform, the movement is detected by the minicomputer and a 12 V peristaltic pump is activated, releasing the solution of interest by a small silicone tube fixed at the side of the yellow platform and connected to an external bottle. On the other hand, when fish cross the "inactive" platform nothing is released into the system, but the number of crossings is measured.

Results & Discussion: For FET, both SCRA tested produced low embryo mortality at the evaluated concentration range. The only endpoint observed was embryos. The non-lethal effects were pericardial edema, loss of posture, and spinal and fin deformity. For the MTC of CHO-4'Me-5'Br-FUBOXPYRA, the increase in the concentration promoted an increase in the absence of movements at 24- and 48-hours post-exposure (hpe) and, at 72 hpe, increase in the general movement. For the MTC of ADB-INACA, the increase in the absence of movements for the higher drug concentration was also noticed, but for all the evaluated periods. As previously demonstrated by Bossé & Peterson (2017), the self-administration protocol is a robust way to assess seeking behavior in adult zebrafish. They demonstrated that adult zebrafish preconditioned with food for five days and then exposed to an opioid delivery system for five days exhibited robust self-administration behavior and also continued to seek the drug despite adverse consequences, in addition to showing signs of stress and anxiety after stopping the drug delivery. In this work, the effects of hydrocodone will be compared to those of two SCRAs, ADB-INACA and CHO-4'Me-5'Br-FUBOXPYRA, on the self-administration behavior of adult zebrafish, allowing the evaluation of the possibility of these drugs causing dependence.

Conclusion: NPS intoxication is an important phenomenon and SCRAs are the most prevalent class reported in seizures or biological determinations. With FET and MTC, the acute toxicity for both drugs were evaluated at embryo and larval zebrafish stages, demonstrating general absence of important effects in the evaluated range. With self-administration assays, it is expected to observe equal or even greater seeking behavior in adult zebrafish exposed to SCRA drugs compared to hydrocodone opioid treatment. With these data it will be possible to clearly understand whether the two SCRAs studied here can achieve reinforcing effects in zebrafish and to assess their potential liability for dependence.

Detection and characterization of N-desethyl etonitazene in a powder sample from a Swiss drug checking service

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NPS P-28

Background & Aims: Recently, 2-benzylbenzimidazole 'nitazene' opioids have emerged on the recreational drug market as new psychoactive substances (NPS). Nitazenes pose a serious threat to public health due to their generally high potency at the μ-opioid receptor (MOR). The increasing number of reports of severe intoxication and overdose deaths associated with nitazenes underscores their high harm potential and stresses the need for close monitoring of this highly dangerous class of new synthetic opioids (NSOs). Drug checking services are harm reduction instances where people who use drugs (PWUD) can have drugs from unregulated markets chemically characterized (e.g., identity and purity of a drug). The PWUD remain anonymous but are obliged to professional counseling, ultimately strengthening informed decision making. Such services are well established in Switzerland and are increasingly recognized to create valuable insights into the recreational drug market, such as early detection of novel compounds and trends. Herein, we report the detection of N-desethyl etonitazene in a powder sample handed in by an individual at a Swiss drug checking service ("Drogeninformation Luzern; DILU", Lucerne, Switzerland) in August 2023. The sample was advertised to contain isotonitazene and was bought through a darknet vendor. However, upon analysis, the sample was found to contain N-desethyl etonitazene, a nitazene analogue that had not yet been formally notified at that time.

Methods: Chemical analyses were conducted to characterize the sample, i.e., nuclear magnetic resonance (NMR), capillary electrophoresis (CE), and high-performance liquid chromatography coupled to high-resolution mass spectrometry (HPLC-HRMS). To gain further information on the MOR activation potential of N-desethyl etonitazene, the powder and a reference standard were investigated using two different in vitro MOR activation assays (i.e., Aequo-Screen® and β-arrestin 2 recruitment assays).

Results & Discussion: NMR and HPLC-HRMS confirmed the presence of N-desethyl etonitazene in the sample at a high purity. Anion analysis by CE showed the presence of chloride anions in the sample, indicating that the obtained sample contains N-desethyl etonitazene in its hydrochloride salt form. N-Desethyl nitazenes have been detected before as metabolites of isotonitazene and etonitazene. However, as first seen with N-desethyl isotonitazene, they are now emerging as standalone drugs. N-Desethyl etonitazene was notified to the European Monitoring Centre for Drugs and Drug Addiction in December 2023, a few months after the sample analyzed in this study was received. In vitro MOR activation profiling revealed a slightly increased efficacy and approximately 6- to 9-fold higher potency of N-desethyl etonitazene compared to fentanyl. N-Desethyl etonitazene showed EC50 values of 3.35 nM and 0.500 nM in the β-arrestin 2 recruitment and Aequoscreen® assays, respectively. In addition, the opioid activity present in the collected sample was evaluated using the bioassays and showed good overlap with the reference standard, in line with the analytical purity assessment. This demonstrates the potential of these bioassays to provide a rapid opioid activity assessment of authentic samples.

Conclusion: As demonstrated with the presented case, drug checking services enable the close monitoring of market developments and trends, rendering them highly valuable for early warning and harm reduction purposes. N-Desethyl etonitazene is the second N-desethyl analogue to emerge on the NPS market, therefore the appearance of other N-desethyl nitazenes is anticipated and should be closely monitored. For forensic toxicological investigations, the emergence of other N-desethyl nitazenes as standalone drugs must be considered to avoid misclassification as metabolites.

NPS P-29

The sudden replacement of etizolam, flualprazolam, flubromazolam use by bromazolam in accidental overdose deaths

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Background & Aims: Bromazolam, a novel designer benzodiazepine (NBD), exhibits potent sedative, hypnotic, and anxiolytic effects, raising concerns regarding its potential for misuse and fatal outcomes, particularly when combined with opioids like fentanyl. Despite limited documented fatalities globally, its use poses a significant threat, exacerbated by underreporting and a lack of routine testing. This study aims to investigate NBD-related deaths in a major U.S. city over a four-year period, utilizing medico-legal death investigations including comprehensive forensic toxicology, pathology, and demographic information.

Methods: San Francisco conducts thorough investigations into all non-natural and sudden unexpected deaths, including the routine testing of over 200 alcohol and drug compounds in decedents under its jurisdiction that includes etizolam, flualprazolam, flubromazolam, and bromazolam analysis. Inclusion criteria encompass accidental overdose deaths involving NBDs in San Francisco from 2020 to 2023.

Results & Discussion: Following eight flubromazolam, 25 flualprazolam and 28 etizolam-related deaths from 2020 to approximately the end of 2022, a sudden surge of 44 bromazolam-related deaths occurred in just a twelve-month period throughout 2023. Bromazolam fatalities frequently involve co-ingestion with opioids, primarily fentanyl, and stimulants like methamphetamine and cocaine. Demographic characteristics indicate a predominance of males,

with a significant proportion lacking fixed addresses. Blood concentrations of bromazolam increased over the study period, suggesting heightened availability and/or purity in the community.

Conclusion: The escalating usage of bromazolam, coupled with its association with fatal outcomes, necessitates heightened awareness among healthcare professionals, public safety agencies, and the community. The findings underscore the urgency for enhanced death investigation, testing, and reporting to facilitate targeted harm reduction strategies for individuals at risk of bromazolam-related morbidity and mortality.

Prevalence of designer benzodiazepines in urine samples from patients included in a Norwegian benzodiazepine substitution study (BMX-BAR)

NPS P-30

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Background & Aims: Benzodiazepine use among people with opioid use disorder is common, ranging between 61–94 % in certain populations. Combined opioid and benzodiazepine use is associated with a lower quality of life and an increased risk of overdose. Bergen Addiction Research Group (BAR) is conducting a multi-center randomized controlled trial for agonist treatment of benzodiazepine dependence (BMX-BAR). The goal is to evaluate the efficacy and safety of substituting patients undergoing opioid agonist therapy (OAT) with diazepam or oxazepam compared to tapering (standard treatment). The main endpoint is the use of illicit benzodiazepines at week 24. Prevalence studies on new psychoactive substances (NPS) use are scarce and even less is known for designer benzodiazepines (DBZD). DBZD accounted for 2 % of the seizures of all benzodiazepines in Norway in 2023, mostly bromazolam. DBZD are often not approved for any medical indication and produced illegally. Consequently, they do not undergo clinical testing and fail to comply with the production standards required for approved pharmaceuticals, and may be contaminated with other drugs. This increases the risk for adverse health events.

The aim of this study is to assess the prevalence of concurrent use of DBZD in a population receiving OAT with a comorbid benzodiazepine addiction.

Methods: The study included adult patients who were undergoing OAT and had concurrent benzodiazepine dependence. The patient population was followed with monthly urine samples and weekly self-reports on illicit use of benzodiazepines at consultations with research staff. Samples were analysed at two laboratories with LC-QTOF-MS. The laboratories had overlapping analytical repertoires with panels that include approximately 60 benzodiazepines and relevant metabolites.

The study was approved in Norway by the Regional Committees for Medical and Health Research Ethics, and by the Norwegian Medicines Agency. All patients provided written informed consent before inclusion.

Results & Discussion: The recruitment period is ongoing, and so far over 500 urine samples have been collected and analysed prospectively from 38 patients included in the project. The preliminary results show detection of at least one DBZD in at least one sample in 24 of the 38 included patients. We detected, for the first time in our region, the DBZD flualprazolam in 4 samples, from 2 different patients, and the metabolite alpha-hydroxyflualprazolam in 9 samples, from 4 different patients. In addition, we found other known and previously detected DBZD such as bromazolam with the metabolite alpha-hydroxybromazolam, in 34 and 29 samples, and etizolam with the metabolite alpha-hydroxyetizolam, in 17 and 37 samples respectively.

Conclusion: This study will help clarify whether and which DBZD are used in the study population. The preliminary results indicate that DBZD should be considered when testing for illicit drug use in OAT patients substituted with benzodiazepines.

Analytical work is performed consecutively, and updated results will be presented at TIAFT 2024.

Separation and quantification of novel synthetic fentanyls in clinical extracts by high pH reverse-phase LC-MS/MS

NPS P-31

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Background & Aims: New psychoactive substances (NPS) represent a significant challenge for analysis within forensic and clinical toxicology laboratories. New fentanyl derivatives have been detected year-on-year since 2012 in the EU and form a part of a wider epidemic issue in the US, with both o-fluorofentanyl and p-fluorofentanyl being detected in post-mortem toxicology samples in the EU and US, respectively, and multiple incidents of o-fluorofentanyl being detected in street samples. Under normal chromatographic and mass spectrometric conditions that may be used by routine drug testing laboratories, newly synthesised fentanyl compounds may be misidentified due to the existence of isomeric and regioisomeric structures of fentanyl derivatives which often coelute and exhibit isobaric behaviour.

While this can cause issues in quantitative confirmation, it as has impact in interpretation for toxicologists and for legal purposes. While one regioisomeric may be classed as a controlled substance, other compounds may not, potentially leading to false prosecution. The aim is to develop a method that can accurately separate and quantify a panel of regioisomeric fluorinated fentanyl compounds.

Methods: Regioisomeric fluorofentanyls (p-fluoro., o-fluoro., m-fluoro., 2'-fluoro., 3'-fluoro., 4'-fluoro., 3-fluoro.), novel synthetic fentanyls, synthetic precursors, metabolites (2-furanylfentanyl, 3-furanylfentanyl, methoxyacetylfentanyl, 4-ANPP, p-Fluoro-4-ANPP), and additional drugs of abuse (heroin, xylazine, fentanyl) were synthesised and characterised by GCMS, NMR, and FTIR.

Whole blood screened positive for fluorofentanyl was extracted by a simple and routine LLE method before evaporation and reconstitution in mobile phase solvents.

The panel was analysed using LC-MS/MS with comparison between different separation methods with variations in pH, and comparisons of reversed phase with HILIC and SFC.

Results & Discussion: A minimum of five MS/MS product ions were generated and selected by automated flow-injection analysis for each compound. The fragmentation of the fluorinated fentanyls in CID MS/MS conditions allow for the spectral separation of regioisomeric groups even with coelution, as one of the most abundant CID fragments of fentanyl generated is N-1-(-phenylethyl) tail, allowing spectral separation from the N-phenyl ring and piperidine. However, this results in overlap of 3-fluorofentanyl with both regioisomeric groups.

The gradient LC methodology utilised a high pH stable C18 column with a mobile phase consisting of MeOH and 0.1% ammonia in water, and here demonstrates the N-1-(-phenylethyl) and N-phenyl ring regioisomers separated with a Rs (min) = 1.3, allowing accurate integration for quantification. While this was selected, low pH reversed phase analysis, as well as HILIC and SFC were also screened and developed. The resolution at low pH was insufficient, causing coelution of m-fluorofentanyl and p-fluorofentanyl, as well as 3'-fluorofentanyl and 4'-fluorofentanyl. HILIC showed full separation of all compounds, excluding 2-furanylfentanyl and 3-furanylfentanyl which were coeluting, but also demonstrated unstable retention times within a reasonable analytical runtime due to the nature of retention with HILIC columns. SFC was screened with four different modifiers and fifteen columns. A 4-minute method was developed and demonstrated separation of all components with Rs (min) = 1.325. This method demonstrated comparatively lower sensitivity than high pH reversed phase, as well as possibly impracticality with developing in routine laboratories due to the additional equipment required to carry out SFC.

This method was then applied to clinical whole blood extracts that were previously screened and tentatively positive for fluorofentanyl by high-resolution mass spectrometry. The validation of this assay (including linearity, accuracy, precision, retention time stability, ion ratios and carryover) was carried out as closely to LAB51 requirements from UKAS.

Linearity demonstrated an R2 of greater than 0.99 for all compounds, with accuracies ranging from 95% to 106% and RSDs of 2.3% to 12.2% across all spiked QCs at different levels (0.25, 15, 75ng/mL). Limits of detection (signal-to-noise greater than 3) and quantification (signal-to-noise greater than 10) were calculated by LabSolutions Insight software, based on the signal-to-noise of the standards analysed, with all compounds giving an LOD less than 0.01 ng/mL or lower and LOQ less than 0.025 ng/mL or lower. The confirmation demonstrated that the fluorofentanyls detected from prior screening in clinical samples show all samples (n = 19) were confirmed positive for p-fluorofentanyl, quantified between 0.44 ng/mL and 31.16 ng/mL, median = 0.91 ng/mL, among other compounds, such as fentanyl (0.45 – 119.46 ng/mL, median = 14.36 ng/mL), xylazine (0 – 14.91 ng/mL, median = 1.42 ng/mL), 4-ANPP (0.12 – 76.62 ng/mL, median = 1.65) ng/mL and p-F-4-ANPP (0.07 – 28.13 ng/mL, median = 0.35 ng/mL). No heroin was detected in samples due to associated metabolic pathways (6-monoacetylmorphine was detected in samples).

Conclusion: The method developed shows a highly accurate, rapid, and efficient method for the analysis of regioisomeric fluorinated fentanyls in biological matrices that could apply to any drug testing lab interested in these compounds. Additionally, with further development, supercritical fluid chromatography shows great promise in equivalent separation and reducing overall cycle time by more than 75%.

Assessment of the ability for five MDMB synthetic cannabinoids to activate the human CB1 receptor *in vitro*

NPS P-32

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Background & Aims: New psychoactive substances (NPS) are chemical substances designed to replicate the effects of traditional drugs, presenting potential risks to both public health and social well-being. The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) closely monitors various classes of NPS, with Synthetic Cannabinoid Receptor Agonists (SCRAs) being the most prevalent. SCRAs are primarily designed to activate the human cannabinoid receptor 1 (CB1), contributing to their widespread use and impact on individuals and communities with known adverse effects. SCRAs typically consists of four parts, namely linked group, core, tail and linker. Five newly emerging SCRAs with MDMB head group (linked group) motif (methyl 3,3-dimethyl-butanoate) where characterised in vitro for their ability to activate the human CB1 receptor. The core was either indole for MDMB-PICA or indazole for MDMB-BUTINACA, MDMB-PINACA, MDMB-INACA and MDMB-MINACA. The tails varied between non-carbon and up to five carbons, whereas the linker motif was the same (carboxamide) for all substances included in the study. The EMCDDA early warnings system have had MDMB-INACA and MDMB- BUTINACA (named MDMB-BINACA in the report) on their watch list since 2022 and 2023 respectively. This study of the MDMB substances, aims to deepen the understanding of the modes of actions and potencies of these recent additions to the NPS market and at the same time as also offer some insights into structure activity relationships.

Methods: Recombinant CHO-K1 cells (AequoScreen®) expressing the human CB1 receptor were used to determine the potency (EC50) and efficacy (Emax) via G-protein coupled signaling using luminescent analysis. Eight different concentrations ranging from 29 pM to 60 µM, were used in at least three independent experiments and JWH-018 was used as a reference. Area under the curve for the luminescence data from each well was determined. EC50 values and Emax with 95% confidence intervals (profile likelihood) and curve fittings (non-linear fit, three parameters) were calculated. The efficacy (derived as the top value from the linear regression) and potency of the substances were compared to JWH-018 using a Brown-Forsyth and Welch ANOVA.

Results & Discussion: Compared to JWH-018 with an EC50 of 26.5 (23.3 – 30.4 95% CI) all of the tested MDMB substances activate the CB1 receptor. Most potent was the indazole with five carbons MDMB-BUTINACA with EC50 at 8.90 nM (7.27 - 10.9 nM 95% CI), followed by the indole with five carbons on the tail MDMB-PICA with EC50 = 11.2 nM (9.81 - 12.8 nM 95% CI). The corresponding indazole with five carbons on the tail MDMB-PINACA exhibited lower potency with a higher EC50 value of 67.6 nM (57.3 – 79.8 nM 95% CI). For the indazoles with zero or one carbon atom in the tail, MDMB-INACA and MDMB-MINACA respectively, a plateau in the in vitro assay was not reached, indicating low potency without a possibility to calculate an EC50 value. Interestingly, all substances exhibited a high maximal activation. Even the substances MDMB-INACA and MDMB-MINACA exhibited an efficacy of at least 101 and 97% respectively, at the highest tested concentration. There was a statistical difference for the efficacy between the reference substance JWH-018 (101 %) and the tested SCRAs that reached a plateau; MDMB-BUTINACA, MDMB-PINACA, and MDMB-PICA at 121, 121 and 118 % respectively (P = <0,0001).

Conclusion: The pharmacological characterization for the activation of the human CB1 receptor, by SCRAs containing the linker MDMB motif, exhibited a wide range of activation, where two SCRAS exhibited both higher potency and efficacy compared to JWH-018. A better understanding of the pharmacological properties of SCRAs can be crucial to estimate the specific risks associated to these substances.

Synthesis and characterization of phenyltropanes: Towards forensic profiling of synthetic cocaine derivatives

NPS P-33

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Background & Aims: Synthetic derivatives of cocaine were originally devised for treating cocaine abuse disorder but are now emerging as a new category of drugs of abuse. This compound class typically encompasses phenyltropanes, 1,4-dialkylpiperazines, phenylpiperidines, benztropines, and dimethocaine, comprising over two hundred substances documented in literature. Currently, most of these substances elude forensic and traffic medical investigations due to the absence of reliable identification and quantification methods. Notably, troparil and dichloropane stand out as phenyltropanes with the highest prevalence on the internet. Similar to cocaine, these substances feature the tropane skeleton, with the (-)-2β,3β-stereoisomers exhibiting the highest psychoactive potential. The synthesis of phenyltropanes invariably yields various stereoisomers and synthetic by-products contingent on the reaction pathway and conditions.

This study aimed to address the lack of reference standards for synthetic cocaine derivatives, their stereoisomers, and primary synthetic by-products. To achieve this, various cocaine derivatives were synthesized via different routes, providing insights into the synthetic pathways preferred by clandestine laboratories in the future.

Methods: Several phenyltropanes and other synthetic cocaine derivatives, along with their stereoisomers, deuterated compounds, and degradation products were synthesized, employing methods outlined in existing literature. Troparil, dichloropane, and other phenyltropanes were synthesized using diverse strategies and conditions to encompass various stereoisomers and potential synthetic by-products. The compounds underwent isolation and purification via flash chromatography and preparative HPLC. Characterization involved NMR spectroscopy, high-resolution mass spectrometry, and polarimetry.

Results & Discussion: All targeted compounds were successfully synthesized and characterized, yielding a spectrum of phenyltropane stereoisomers and deuterated compounds that serve as reference standards for developing identification and quantification methods in seized samples and biological specimens. In addition, disparities in the efficacy and practicality of synthetic routes were evaluated, enabling preliminary insights into the routes favored by clandestine labs for producing synthetic cocaine derivatives. Notably, the presence of 2α,3β-stereoisomers may stem from phenyltropane synthesis via Grignard reaction, while 2β,3α-stereoisomers could result from synthesis via Suzuki coupling.

Conclusion: The synthesis and characterization of multiple phenyltropanes as reference substances represent a significant stride toward forensic investigation of synthetic cocaine derivatives. Moreover, isolating and characterizing synthetic by-products and distinct stereoisomers will facilitate the development of specific methods for comprehensive drug profiling, offering insights into the quality of substances retailed on the internet and the production techniques employed in clandestine labs.

NPS P–34

Emergence of 2-MMC in tvhe French Alps: Description of 5 clinical and forensic toxicology cases

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Background & Aims: Until 2010, the most commonly available cathinones on the European market were mephedrone (4-MMC) and methylone. In 2022, 3-methylmethcathinone (3-MMC) and 3-chloromethcathinone (3-CMC) were the two most seized synthetic cathinones in Europe, reflecting their current widespread availability (EMCDDA). The aim of this study was to present the emergence of 2-methylmethcathinone (2-MMC) from November 2023 to January 2024 in the French Alps, based on 5 laboratory documented cases.

Methods: To document cases of 2-MMC exposition from different toxicological contexts, 50 µL plasma, blood and urine samples were prepared using one-step SPE extraction on an Ostro® plate (Waters). For oral fluid (theoretical 350µL) on dry FLOQSwab®, preparation was based on a phosphate extraction buffer followed by deproteinisation with acidified glacial acetonitrile. Both types of extract were analysed using UPLC-MS/MS. Chromatographic separation was carried out on an Acquity UPLC HSS C18 polyvalent column (2.1mm x 100mm) and with a 15-minute one-dimensional chromatography. Tandem mass spectrometry analysis was performed on a Xevo TQ-XS mass spectrometer (Waters). The transitions used for X-MMC were: 178>91 ; 178>145 ; 178>160. The limit of quantification for 2-MMC was 1 ng/mL and the method was linear up to 500 ng/mL for oral fluid, plasma and urine. Dilutions were made if necessary. An evaluation of isomer resolution by LC-HRMS on an Accucore Phenyl-Hexyl column (2.1mm x 100mm) with a 15.5-minute run and an Exploris 120 (Thermo Scientific) was also performed. Immunoassay screening for MDMA and amphetamine using EMIT kits on Atellica CH Analyzer (Siemens Healthineers) with a 300 ng/mL cut off was carried out on urine.

Results & Discussion: Concerning the resolution of the three isomers in UPLC-MS/MS, the retention times for 2-MMC, 3-MMC and 4-MMC were 2.98 min, 3.16 min and 3.12 min respectively. In LC-HRMS, 2-MMC and 4-MMC were co-eluted (3.33 min and 3.34 min) while 3-MMC was separated (3.39 min).

In a case of drug-facilitated sexual assault (DFSA) involving a 32-year-old man with amnesia and a road accident, 2-MMC (2012 ng/mL) and 3-CMC (466 ng/mL) were detected in urine 48 hours after the incident. Urine immunoassays for MDMA and amphetamine were positive.

In a case of attempted autolysis with malaise and convulsions in a 39-year-old man due to GHB and 2-MMC intoxication (reported by the patient), 2-MMC (106 ng/mL and >10000 ng/mL) and GHB (40.8 mg/L and 2458 mg/L) were measured in the plasma and urine 3 hours after the admission. MDMA and amphetamine urine tests were positive.

As part of an investigation into the cause of death in the suicide of a 28-year-old man who had fallen from a cliff, 2-MMC was measured at 4.7 ng/mL in peripheral blood (estimated) and 418 ng/mL in urine. The urine immunoassays were negative.

In two cases of driving under the influence of drug (DUID), positive oral fluid roadside tests for amphetamines led to the identification of 2-MMC in oral fluid at concentrations above 1000 ng/mL (estimated at 9969 and 2864 ng/mL).

Conclusion: Cathinones can be found in most toxicology contexts: voluntary intoxication, DFSA, DUID and post-mortem. A simple and common UPLC-MS/MS method is sufficient to identify the ortho isomer of methylmethcathinone. In Switzerland, the drug checking system nuit-blanche.ch also reported 2-MMC in powders in 2023. Monitoring of 2-MMC in France is ongoing.

Examining in-sample stability of synthetic cannabinoid biomarkers in municipal wastewater

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Background & Aims: Municipal wastewater comprises a wide range of chemicals excreted by individuals after abusing various illegal substances. Wastewater-based epidemiology (WBE), which analyzes municipal wastewater influents, is becoming increasingly popular as a valuable source of information on a given community for estimating collective drug usage. The WBE approach serves as a valuable complementary tool to current epidemiological methods. Yet, the accuracy of consumption estimates hinges on several uncertainties; not only on the accuracy of the concentration measurements conducted by analytical techniques but, among other things, on the stability of the selected urinary biomarker during the sample collection and storage as well as during time in a sewer system. The stability of traditional illicit substances in wastewater has been investigated extensively, but new psychoactive substances (NPS), which are increasing in number and the dangers they pose, have taken a back seat. Studies of synthetic cannabinoids, on the other hand, the largest group of NPS monitored by international bodies, have been limited due to the analytical challenges posed by the substances. With this study, a systematic investigation was conducted to assess the variability of 20 synthetic cannabinoid biomarkers' in-sample stability to determine the optimal pretreatments, temperature, and time intervals for storage.

Methods: Four different 24-h composite influent wastewater samples from 2 wastewater treatment plants were collected in high density polyethylene bottles. Twenty synthetic cannabinoids, CUMYL-4-CN-B7AICA, ADB-FUBINACA, 5F-APINACA, 5F-APINACA-N-4-hydroxypentyl metabolite, XLR-11, XLR-11-4-hydroxypentyl metabolite, AM-2201, AM-2201-4-hydroxypentyl metabolite, AM-2201-6-hydroxyindole metabolite, 4F-MDMB-BINACA, 4F-MDMB-BINACA-butanoic acid metabolite, 5F-MDMB-PINACA, 4F-MDMB-BUTICA, 4F-MDMB-BUTICA-N-4-hydroxybutyl metabolite, 5F-ADBICA, 5F-EMB-PICA-N-5-hydroxypentyl metabolite, 5F-BZO-POXIZID, BZO-HEXOXIZID, MDMB-4en-PINACA and ADB-BUTINACA were selected from latest reports of international organizations. The reference standards of each targeted analyte were diluted to 1 µg/mL stock solution as a mixture. The stock mixture of the isotope-labeled internal standards (ILISs) of XLR-11-d5 and 5F-MDMB-PICA-d5 at 1 µg/mL concentration was also prepared. On the initial day of sample collection, 50 mL of the fresh wastewater sample (pH ≈ 7) was transferred into polypropylene-screw cap centrifuge tupes. Then sample was spiked with selected reference standards to ensure the final concentrations were 0.05 µg/L, higher than potential residual levels, and vortexed. The stability of the analytes was investigated without any pH adjustment by keeping the samples at different temperatures (-20°C, 4°C, and 20°C) for different time intervals (24h, 48h, 7d, and 30 days). Thirty-day stability was tested only in the freezer (-20°C) considering the possibility of long-term storage.

A centrifugation step (5 min 8000 rpm) was conducted to avoid possible filtration losses due to lipophilic nature of the substances prior to extraction. A fully validated sample preparation procedure of solid-phase extraction (Oasis HLB cartridges) followed by the liquid chromatography-tandem mass spectrometry method was utilized. Elution was conducted with 3 mL of methanol, and the eluates were evaporated to dryness before reconstruction with 500 µL mobile phase mix (1:1, v/v of 2mM ammonium formate and 0.1% formic acid in water/ 0.1% formic acid in methanol). For each parameter, 3 different samples and one blank sample, were studied and each was analyzed in 3 replicates. Samples were analyzed in positive electrospray ionization mode and the total run time was 17 min.

Results & Discussion: All results were normalized to t = 0 and calculated as recovery %. The evaluation considered recovery between 80% and 120% as highly stable, between 80%-40% as moderate, and <40% as low stability.

A thorough examination of existing literature revealed that this study represented the first investigation into the stability of substances such as CUMYL-4CN-B7AICA, 4F-MDMB-BINACA-N-4-hydroxybutyl metabolite, 4F-MDMB-BINACA butanoic acid metabolite, 5F-BZO-POXIZID, and BZO-HEXOXIZID in wastewater. The findings indicated that synthetic cannabinoids within this scope exhibited greater stability when stored at +4°C and -20°C compared to room temperature. Most substances retained their stability for up to 48 hours, although exceptions were noted for 5F-BZO-POXIZID, BZO-HEXOXIZID, and AM-2201-6-hydroxyindole for all temperatures. However, it's important to note that long-term storage at any temperature is not advisable for most synthetic cannabinoids. The study concluded that 5F-BZO-POXIZID and BZO-HEXOXIZID began to degrade significantly within 24 hours at all temperatures, suggesting that monitoring these substances as biomarkers in wastewater might not be suitable for extended sampling periods of 7 consecutive days or more. However, additional research is needed to investigate whether preservation methods like acidification or sodium metabisulfite could improve their stability, as seen in certain other compounds. Additionally, it is recommended to acquire the reference standard of previously identified urinary metabolites of these substances (if available) and conduct stability studies with those metabolites to determine more stable biomarkers in wastewater.

Conclusion: Determining the in-sample stability of biomarkers throughout sample collection and storage settings is a concern that must be addressed in a quantitative wastewater study to obtain valid results, particularly when investigating compounds such as NPS with low concentrations. This study allowed us to discuss the biomarker potential of some up-to-date synthetic cannabinoids in WBE and revealed their optimal storage conditions for accurate consumption estimations.

NPS P-36

Construction and application of a rapid high throughput screening method for 270 SCs and metabolites based on LC-Q-TOF-MS/MS

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Background & Aims: Synthetic cannabinoids (SCs) constitute one of the largest groups of new psychoactive substances and become popular recreational drugs of abuse for their psychoactive properties. The continuous transformation of SCs also leads to an endless emergence of new types. In July 2021, China put into effect a new generic legislation banning SCs containing one of seven general core scaffolds. An efficient, high-throughput screening method is therefore very important for their identification. The aim of this study is to develop a high-resolution mass spectrometry screening method for simultaneously screening 270 SCs and metabolites in blood and urine and summarize the fragmentation ion cleavage rules through the established HR-MS/MS spectral library, providing reference for non-targeted screening of unknown SCs.

Methods: Blood and urine samples were analyzed after protein precipitation (acetonitrile: methanol=1:1) and centrifugation by UHPLC-QTOF-MS/MS. A 19-minute gradient on Kintex Biphenyl column (3 x 100 mm, 2.6 µm, 100 Å) was employed to ensure good separation. The column temperature was set at 40 °C, and the flow rate was 0.4 mL/min. The composition of the mobile phase was 0.1% formic acid in water and 0.1% formic acid in methanol. The MS source conditions were as follows: curtain gas (CUR), 35 psi; collision gas (CAD), 7; nebulizing gas (GS1), 40 psi; heater gas (GS2), 70 psi; ion spray voltage (IS), 3500V in positive mode; source temperature, 500°C. The mass acquisition was performed using information-dependent acquisition (IDA) that consisted of a survey scan and dependent product ion scan in a single run. The survey scan was performed in a full-scan time-of-flight MS (TOF-MS) between m/z 100 and 1000 with DP and collision energy (CE) at 30 V and 10 eV, respectively. The IDA-MS/MS was performed under the following conditions: MS/MS threshold, 100 cps; ion tolerance, 50 mDa; CE was ramped over an interval by entering a CES value, and the CE and CES were set at 35 and 15 eV, respectively (i.e., 35 ± 15 eV).

Results & Discussion: The HR-MS/MS spectral library encompasses comprehensive details on 270 compounds, including CAS numbers, original molecular formulas, in-source fragment formulas, and retention times, covering seven primary core scaffolds of synthetic cannabinoids (SCs) banned in China, as well as the unregulated novel OXIZIDs, among others. Under optimal chromatographic conditions, baseline separation for most isomers can be achieved. Method validation demonstrated detection limits (LODs) at which all 270 compounds were identified at a concentration of 10 ng/mL in both whole blood and urine samples. In whole blood, 265 compounds (98.1%) exhibited recovery rates above 85%, while 154 compounds (57.0%) showed matrix effects exceeding 80% at the lowest concentration tested (10 ng/mL). In urine, 193 compounds (71.5%) had recoveries surpassing 85%, and 248 compounds (91.8%) displayed matrix effects greater than 80% at low concentrations (10 ng/mL). Some substances exhibited matrix effects over 100%, potentially due to ion enhancement effects. Nevertheless, matrix effects did not exceed 120%, and the relative standard deviation (RSD) values for matrix effects across four different concen-

trations during methodological verification were below 15%. These findings meet the requirements for methodological verification. Therefore, ion enhancement does not impair the qualitative results.

We identified thirty-nine compounds with relatively low potency, requiring a concentration of 10 ng/mL to achieve satisfactory peak and fragment ion detection. These compounds are presumed to undergo in-source fragmentation due to the presence of primary amino groups in their indole or indazole core structures. In contrast, twenty-three compounds can effectively utilize in-source fragmentation. For example, ADB-BICA (m/z 364.2019) was screened using in-source fragmented ions (m/z 347.1654) resulting from the loss of an amino group. This approach effectively reduces interference from hybrid peaks and matrix components, thereby facilitating screening analysis. Based on this phenomenon, we also employ source fragmentation as a tool for parent ion screening. In-source fragmentation offers enhanced sensitivity and minimizes false positives.

Conclusion: We successfully developed a simple, reliable, and highly efficient LC-Q-TOF HRMS method based on efficient mass resolution and an accurate database for simultaneous screening of 270 SCs and metabolites in blood and urine samples. The method can automatically complete the comparison of mass errors, isotopic distributions, retention times and MS/MS spectra between the suspected target and the library and was successfully applied to detect SCs in authentic samples. We also summarized some fragmentations of SCs and in-source fragmentation which will be helpful for forensic laboratories seeking to identify and structurally analyze these types of compounds.

Unusual co-detection of xylazine, nitrazolam, bromazolam, and nitazenes in multiple clinical cases.

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Background & Aims: Xylazine is a drug of concern, it has seen increased prevalence, in combination with opioids, in illicit drug overdoses in the United States and some recent detections in Europe, however it has not yet been reported in Australia in the context of illicit drug use. Similarly, benzimidazole synthetic opioids, 'nitazenes', are increasingly being detected, including in Australia, and are of concern due to their high potency. Novel benzodiazepines have been seen for some time however with the popularity of the specific benzodiazepines constantly changing. The Emerging Drugs Network of Australia (EDNA) is a national toxicosurveillance program which records clinical toxicity with analytically confirmed results.

In this report a cluster of cases is presented in which the same combination of drugs was detected in hospital presentations across South Australia (SA), as part of the EDNA program. This unique drug combination has not been detected, to our knowledge, in Australia previously.

Methods: To identify drugs involved in Emergency Department (ED) presentations, blood samples are taken from patients with presumed illicit drug intoxication and requiring intravenous access/blood testing as part of their routine care after presentation to SA's four major EDs, as part of the nationwide EDNA toxicosurveillance program. Blood samples were analysed at Forensic Science SA using a comprehensive toxicological screening process including an alcohol screen, an alkaline supported liquid extraction (SLE) and an acidic SLE with analysis by liquid chromatography time-of-flight mass spectrometry (LC-QTOF). A library of approximately 700 drugs was used for screening and detection based on retention time, accurate mass and MSMS spectral data.

Results & Discussion: Toxicological analysis of EDNA blood samples identified the presence of xylazine, nitrazolam, bromazolam, protonitazene and metonitazene in four hospital admission samples over a 10 day period. All patients survived. Patient information indicated they all had injected heroin, arrived by ambulance and were triaged at ATS 1 (i.e. immediate care required). Clinically, all were sedated (Glasgow Coma Scale 3), showing hypoventilation with minimum respiratory rates of 0-4 breaths per minute. Naloxone was administered in all cases. In addition to the 5 NPS drugs mentioned, morphine was detected in all four cases and methylamphetamine in three. The short time period and severity of the presentations combined with the unique drugs present resulted in an alert being issued to the relevant health agencies.

Internationally there are numerous reports of xylazine and/or nitazenes being used as an adulterant in heroin, with and without users' knowledge, but until recently this has not been an issue in Australia. The knowledge that these potent drugs are now present in the local illicit drug supply is important for prevention of drug harm and in treatment settings, particularly due to naloxone not being effective in the treatment of xylazine overdoses.

Conclusion: Analytical confirmation of drugs present in Emergency Department presentations is vital to ensure a clear picture of the drugs causing harm in the community. These are the first documented cases of this combination of drugs and the first detections of nitrazolam and xylazine in SA. Further illustrating the value of EDNA as an early warning and surveillance system and critical to Australia's strategic approach to reduce drug-related harms.

NPS P-38

Results of identification of new psychoactive substances using gas chromatography-mass spectrometry

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Background & Aims: New types of psychoactive substances are attracting a lot of attention worldwide, and their use is likely to increase among young people who do not know enough about the dangers of these substances. In Mongolia, a new type of psychoactive substance was registered for the first time in 2023. In our country, it is believed that the domestic production of new types of psychoactive substances is not registered, and it is illegally distributed across the country's borders.

New types of psychoactive substances are often available in liquid and oily form. Common types are:
1.MDMB-4en-PINACA
2. ADB-BUTINACA.
Gas chromatography-mass spectrometry (GC-MS) was used to identify new types of psychoactive substances.

Methods: 2.Methods: 2.1. Necessary equipment, small tools, reagents: Gas chromatography-mass spectrometry GC/MS, GC/MS sample glass container vial, filter,1 disposable syringe, eppendorf tube, vortex, standard substance (MDMB-4en-Pinaca), HPLC grade hexane, methanol.

2.2. Sample preparation:
Blank and positive control samples should be prepared for each test batch and analyzed with samples. Take 0.5 ml of the oily liquid and put it in an eppendorf tube, add 0.5 ml of HPLC grade methanol to extract. Use a disposable syringe under vortex, filter it and make a GC/MS-vial. Methanol was used as a blank sample.

Results & Discussion: 3. GC/MS analysis (Results discussion):

3.1. GC/MS equipment conditions:
In our research, we used an Agilent 7820A GC/ 5977E MSD gas chromatography-mass spectrometer.

GC Columns: HP-5MS UI (30 m length, 0.25 mm diam, 0.25 µM film Agilent technologies JW GC columns), oven temperature: 60-280 degrees, analysis term: 24.667 minutes, carrier gas: Helium (flow: 1 mL/min, pressure 8.2 psi).

3.2. From October 2023 to March 2024, the Chemical laboratory of the National Forensic Agency of Mongolia analyzed the evidence of 17 cases. NIST, Cayman spectral library, AMIDS, and SWGDRUG libraries were used to process the analysis results. The retention time, chemical formula, molecular weight, molecular ion [M+], exact mass [M+H]+ were used to detect synthetic cannabinoids.

A total of 17 case samples were analyzed: Usually yellow, sometimes colorless, liquid, oily, was fragrant.

№	Total detected cases	Name of detected substance	RT (min)	Proportion of total organic mass
1.	9	ADB-BUTINACA	19.590±0.2	6.181%-15.067%
2.	8	MDMB-4en-PINACA	18.468±0.2	7.357%-14.698%

Conclusion: 1. New types of psychoactive substances found in Mongolia are in liquid and oily form, and the most common types are MDMB-4en-PINACA and ADB-BUTINACA.

2. The proportion of total organic mass in the supplied liquids is 6.181%-15.067% for ADB-BUTINACA and 7.357%-14.698% for MDMB-4en-PINACA.

Detection of novel synthetic cathinones derivatized with PFPA using gas chromatography/mass spectrometry

NPS P–39

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Background & Aims: Novel psychoactive substances (NPS) have proliferated globally under various names such as "designer drugs" and "legal highs". NPS are readily obtainable online and frequently substituted with slightly modified variants in an attempt to circumvent regulatory measures. According to the UNODC Early Warning Advisory (EWA) on NPS in 2023, stimulants account for a significant proportion of abuse drugs, representing 35 %. The trend of abuse drugs is influenced by various factors, such as social conditions, regulations, and market liquidity in regions and countries, which can be sufficiently replaced by other types of drugs. Synthetic cathinones as one of the 11 groups classified as NPS by the United Nations Office on Drugs and Crime (UNODC) are derivatives of the naturally occurring compound cathinone in the khat plant Catha edulis. Similar to amphetamines, synthetic cathinones are psychomotor stimulants that exert their effects by imparing the normal function of plasma membrane transporters for dopamine, norepinephrine, and 5-HT. The determination of synthetic cathinones can be challening due to the limited information available on new substances and the difficulty in obtaining standards for seized materials, and due to the detection of similar parent drug structures and unidentified metabolites for biological samples of drug abusers. Therefore, international cooperation is imperative.

Currently, the National Forensic Service utilizes gas chromatography/mass spectrometry (GC/MS) to screen for psychoactive substances in seized materials and biological samples from abusers, which has advantages in selectivity, sensitivity, robustness, and an extensive database. Furthermore, derivatization can enhance the resolution of substances exhibiting structural similarity or isomers. This study aims to facilitate the indentification of unknown cathinones through structural analysis of ten novel synthetic cathinones derivatized with pentafluoropropionic anhydride (PFPA) using GC/MS.

Methods: A 50 µL of 100 mg/L standard solution of S-cathinone, 2R-methcathinone, hexedrone, mexedrone, N-ethylhexedrone, N-ethylheptedrone, methylone, ethylone, eutylone, N-ethylpentylone, and eutylone-D5(internal standard) were added to the test tube, respectively. The samples were evaporated under nitrogen with a 50 µL of 1 % hydrochloride in methanol to prevent volatilization, and then derivatized by adding of 50 µL of dehydrated ethyl acetate and 30 µL of PFPA. The residues were incubated for 15 min at 65 °C, cooled, and then evaporated to dryness under nitrogen before being reconstituted with 100 µL of dehydrated ethyl acetate. The GC/MS system consisted of a Hewlett Packard 7693A autosampler, HP 7890B GC system, and GC-MSD 5977 mass selective detector. The column was a fused silica capillary column (HP-5MS capillary column, 30 m×250 mm×0.25 mm). The injector was operated in the splitless mode, the injection volume was 2 µL, the injector temperature was 250 °C, and the transfer line temperature was 280 °C. Initial oven temperature was 100 °C, maintained for 1 min, increasing at 15 °C/min to 160 °C, and increasing at 30 °C/min to 280 °C. GC/MS operated in scan mode range of m/z 50~450.

Results & Discussion: To identify the structures of synthetic cathinones with a 2-aminophenone backbone of low molecular weight, sufficient retention time separation through PFPA derivatization using GC/MS was required. By considering the structural features of alkyl groups in synthetic cathinones, it became possible to identify regular fragment ions. Confirming the exact mass of parent substances was crucial due to the formation of analogous fragment ions. The PFPA-derivatized cathinones exhibited sufficient selectivity, and the fragment ion patterns of structurally related substances were significant.

Conclusion: This study examined the PFPA-derivatized structures of ten synthetic cathinones with a 2-aminopropiophenone backbone and proposed the feasibility of simultaneous GC/MS analysis. The regular pattern of fragment ions obtained from this research is anticipated to facilitate the rapid structural prediction of unknown cathinones.

Using a virtual liquid chromatography tool to develop methods for novel psychoactive substances

NPS P–40

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Background & Aims: Novel psychoactive substances (NPS) have created a challenge for toxicology laboratories. New NPS are constantly disappearing as fast as they emerge, making it difficult to stay on top of which compounds are necessary to add to laboratory testing scopes. The development and optimization of liquid chromatography (LC) separations is time consuming and costly, often requiring several steps including literature research, column

selection, method scouting, method development, and method optimization. To alleviate the burden of sacrificing instrument-uptime, labor and materials, an instrument-free software modeling tool was developed to include a comprehensive drugs of abuse (DoA) library. Restek's Pro EZLC (https://ez.restek.com/proezlc) is a free online tool that allows users to obtain optimized separations while maintaining critical pair resolution by adjusting parameters such as column dimension, mobile phase, gradient programs, and more for almost 300 compounds including the 38 newly added NPS drugs.

The primary objective of this study is to use a chromatographic modeling tool to develop effective LC-MS/MS methods for various NPS compounds including synthetic opioids, designer benzodiazepines, synthetic cathinones, synthetic cannabinoids, and toxic adulterants.

Methods: The NPS library utilized the same design space as the existing DoA library. Retention times were collected using method conditions consisting of a fast (5 minute) and slow (15 minute) gradient, 30°C/60°C temperature points, and ACN/MeOH mobile phases on Raptor Biphenyl and Raptor C18 columns in a 50 x 2.1, 2.7 µm dimension. The 38 NPS compounds were divided into three small groups to account for the separation of isobars and to generate the optimal points per peak for instrument analysis. A set of 8 compounds, referred to as "meld compounds", were then added to each group. These meld compounds spanned the chromatographic space and were used to verify instrument performance from injection to injection. Data was collected and input into the platform. Results of retention times between experimental and modeled data were compared. To verify the ability of the modeler to develop methods for NPS, three methods were developed and optimized using the chromatogram modeler for the following NPS subclasses: 1) synthetic opioids and toxic adulterants 2) designer benzodiazepines 3) stimulants and synthetic cannabinoids. All methods utilized a Raptor Biphenyl 100 x 2.1, 2.7 µm column with a MPA of water and MPB of methanol, both acidified with 0.1% formic acid. The flow rate was 0.6 mL/min and the column temperature was 40°C. The developed methods were transferred to an LC-MS/MS system and the experimental results were compared with the modeler.

Results & Discussion: The online chromatogram modeling tool successfully developed methods for NPS compounds. Developing the methods using the virtual chromatography tool was completed in under ten minutes per method. The acceptance criteria for retention time agreement between experimental and modeled values was set at +/- 15 seconds, chosen to represent a typical MRM window. All analytes in all three methods fell within this window, as well as maintaining elution order and resolution. For example, Isotonitazene had a predicted retention time of 2.86 minuntes and an experimental retention time 2.75 minutes, for a difference of 6.6 seconds. Eutylone had a predicted retention time of 4.42 minutes and an experimental retention time of 4.18 minutes, for a difference of 14.4 seconds. Based on the acceptance criteria as defined, each NPS method was successfully transferred from the virtual model to an LC-MS/MS instrument.

Conclusion: As NPS continue to proliferate the illicit drug market, the burden of adding these compounds to laboratory testing scopes becomes the obligation of LC method developers. Utilizing tools such as a virtual chromatography modeler can help method developers deal with the challenges these emerging compounds present.

NPS P–41

Dipyanone, a new methadone-like synthetic opioid: Metabolism study using human hepatocytes and postmortem urine

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Background & Aims: In the last decade, illicit fentanyl analogs and other novel synthetic opioids (NSOs) have caused thousands of fatalities worldwide, pressing for reinforced legislation and prevention. New NSO subclasses have emerged on the illicit drug market to fill the vacuum left by the recent laws governing drug control.

Dipyanone, also known as N-pyrrolidino-methadone, is a synthetic opioid with analgesic and spasmolytic effects that is structurally related to methadone, dipipanone, and phenadoxone, three substances internationally controlled under the Schedule I of the 1961 UN Single Convention on Narcotic Drugs. In vitro studies have shown that dipyanone exhibits an analgesic potency similar to that of methadone, i.e., lower than that of many fentanyl analogs or recently marketed NSOs such as the 'nitazenes'. However, it has been involved in several fatalities in Germany and Slovenia since its first detection in 2021. Due to the risks of respiratory and central nervous system depression, identifying dipyanone exposure through the detection of the parent drug and/or specific metabolites may be critical in analytical toxicology. However, little is known about dipyanone, let alone its metabolism. To address this concern, we aimed to investigate dipyanone metabolic fate in humans to identify specific biomarkers to help document exposure in clinical and forensic casework and set the basis for further pharmacokinetic studies.

Methods: Dipyanone reference standard (10 µmol/L) was incubated for 3 h with 106 pooled human hepatocytes/mL in 20 mmol/L HEPES in Williams' medium E. Incubates were analyzed by liquid chromatography-high-resolution tandem mass spectrometry (LC-HRMS/MS) with a separation on a biphenyl-bonded analytical column (150 x 2.1 mm, 2.6 µm) and detection in full-scan MS and data-dependent MS/MS with a Q Exactive (Thermo Scientific), and raw data were screened with Compound Discoverer (Thermo Scientific). Analyses were supported by metabolite predictions in humans with GLORYx open-access software (Hamburg University, Germany).

Urine specimens from two dipyanone-positive fatalities that occurred in Germany in 2021 were analyzed under similar conditions with and without enzymatic glucuronide hydrolysis. In the first case, dipyanone concentration was 5,500 and 80 ng/mL in urine and blood, respectively; mitragynine, amphetamine and ketamine derivatives as well as their metabolites were also detected. In the second case, dipyanone concentrations were 1,000 and 720 ng/mL, respectively, with no other compound of toxicological interest being detected.

Results & Discussion: A total of 14 metabolites were found in the incubates. Additionally, 8 phase I and phase II minor metabolites were detected in the two urine specimens. Both in the incubates and authentic samples, the most intense metabolites were produced through pyrrolidine ring opening to the corresponding N-butanoic acid or N-butanol and further cyclization, similar to the transformation occurring from methadone to 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), its main metabolite. Other reactions included hydroxylation, reduction, O-glucuronidation, O-sulfation, and combinations thereof. Dipyanone itself was detected with an intensity approximately twice higher than that of the main metabolite in both urine specimens. Glucuronide hydrolysis did not substantially increase the signal of the major metabolites.

Conclusion: For the first time, dipyanone human metabolism was assessed using an in vitro model and confirmation with authentic postmortem cases. We propose the parent drug and the two main metabolites formed after pyrrolidine ring opening to N-butanoic acid or N-butanol and cyclization as biomarkers of exposure. Good correlation was found between dipyanone incubates and authentic urine samples, and human hepatocyte incubation resulted to be a suitable model for the prediction of dipyanone metabolic profile. Considering the never-ending emergence of NSOs on the illicit drug market and the health risks associated with NSO use, comprehensive metabolite identification studies are crucial for their identification in authentic casework.

α-Pyrrolidinoisohexanophenone (α-PIHP) in three fatal cases

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NPS P-42

Background & Aims: The New Psychoactive Substances (NPSs) are widely spread worldwide through the illicit markets and are increasingly the cause of intoxication deaths. Alpha-pyrrolidinoisohexanophenone (α-PiHP) is a positional isomer of α-pyrrolidinohexanophenone (α-PHP) and has been reported for the first time in 2016 at seized materials in China. Both substances are pyrovalerone derivatives from the synthetic cathinones group, and mainly exert cardiovascular, psychological, and neurologic effects. In addition, as published in literature and regardless of their isomerism, both cathinones can appear separately or together in a mixture. This work presents three forensic fatal cases associated with the consumption of α-PiHP and/or α-PHP: case 1 involves a 41-year-old man, drug user, mostly of "bloom", who committed suicide by hanging; case 2 reports to a 32-year-old man, addicted to synthetic drugs, associated with a sample of "weed" collected next to the corpse; case 3 concerns to a 58-year-old male drug addict, who died after being admitted to the emergency department in a state of coma.

Methods: The toxicological analyses were carried out in peripheral blood in the three cases and, in case 2, the sample of "weed" was also analysed. Samples (500µL) were prepared with 0.1 M phosphate buffer, extracted by solid-phase extraction and analysed through gas chromatography coupled with mass spectrometry (GC-MS) in full-Scan monitoring mode to search for unknown substances. Subsequently, the samples were analysed in single-ion monitoring mode to confirm the substances detected in full-Scan. The ions 140, 98 and 77 were monitored for α-PiHP; 140, 105 and 77 for α-PHP; and 185 for cocaine-d3 (internal standard).

Results & Discussion: In case 1 was detected and confirmed α-PiHP. Other substances were also detected and confirmed such as tramadol, fluoxetine, diazepam, nordiazepam, cyamemazine, olanzapine, paliperidone and risperidone. Regarding case 2, in the "weed" we were able to detect several cannabinoids (cannabicyclol, cannabichromene, cannabidiol, cannabidivarol, cannabigerol, cannabinol, delta-9-tetrahydrocannabinol, delta-8-tetrahydrocannabinol and tetrahydrocannabivarin), nicotine and α-PiHP. In peripheral blood was confirmed α-PiHP, cocaine and its metabolites (benzoylecgonine and ecgonine methyl ester), delta-9-tetrahydrocannabinol and 11-nor-9-carboxy-delta-9-tetrahydrocannabinol, phenacetine (a cocaine cutting agent), morphine, paracetamol, alprazolam, nordiazepam, sertraline and mirtazapine. In case 3, both α-PHP and α-PiHP were detected and confir-

med in peripheral blood, such as paracetamol. Therefore, the toxicological results of the three cases are relevant to complement the case histories since the presence of α-PiHP in the peripheral blood can be related with the death of individuals.

Conclusion: With the three cases presented in this work, we can conclude that the search for NPS in biological and non-biological specimens plays an important role, especially in cases of drug-related deaths. The concomitant use of traditional drugs of abuse, prescription medication, α-PHP and α-PiHP, either separately or in combination, increases the possibility of a fatal intoxication since both substances have a high toxic potential.

NPS P-43

Detection of xylazine in whole blood samples using high-resolution mass spectrometry

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Background & Aims: Xylazine is an α2 adrenergic receptor agonist originally designed for veterinary purposes as a tranquilizer and anesthetic that is not classified as a controlled substance under the Controlled Substances Act and as a result not approved for human use. In recent years, xylazine has emerged as a human health treat among people using drugs of abuse. Poly-drug intake, such as the addition of xylazine to synthetic opioids, such as fentanyl, significantly increases the risk of overdose. Individuals using illicit drugs might not be aware of the presence of non-opioid substances, such as xylazine, in drug products. Given the global concerns about the role of xylazine in opioid overdoses, it is essential to employ a comprehensive drug screening approach. SWATH DIA is a valuable tool for this purpose as it collects MS/MS fragment data on all features present in a sample. This untargeted screening approach provides information to aid in identifying and quantifying known and emerging drugs and adulterants in toxicology samples.

Methods: Discarded postmortem authentic human whole blood samples known to contain xylazine were provided by the Center for Forensic Science Research and Education. These samples were aliquoted (0.5 mL), pretreated with 3 mL of phosphate buffer (pH 6) and centrifuged at 3000 rpm for 10 minutes. The samples were loaded onto a solid phase extraction (SPE) cartridge consisting of a C8 and ion exchange phase (benzenesulfonic acid) bonded to the same particle. Cartridges were conditioned with methanol and water, followed by equilibration with 1 mL of phosphate buffer. After sample loading, the cartridge was washed with water, followed by 0.1M HCl, then methanol, before drying under vacuum. The analytes were eluted using 2 aliquots of 1.5 mL of 78:20:2 dichloromethane/isopropyl alcohol/ammonium hydroxide. Finally, 100 µL of 90:10 methanol/HCl solution was added, and the sample was dried under a nitrogen stream and reconstituted to 200 µL in 95:5 water/acetonitrile.Discarded postmortem authentic human whole blood samples known to contain xylazine were provided by the Center for Forensic Science Research and Education. These samples were aliquoted (0.5 mL), pretreated with 3 mL of phosphate buffer (pH 6) and centrifuged at 3000 rpm for 10 minutes. The samples were loaded onto a solid phase extraction (SPE) cartridge consisting of a C8 and ion exchange phase (benzenesulfonic acid) bonded to the same particle. Cartridges were conditioned with methanol and water, followed by equilibration with 1 mL of phosphate buffer. After sample loading, the cartridge was washed with water, followed by 0.1M HCl, then methanol, before drying under vacuum. The analytes were eluted using 2 aliquots of 1.5 mL of 78:20:2 dichloromethane/isopropyl alcohol/ammonium hydroxide. Finally, 100 µL of 90:10 methanol/HCl solution was added, and the sample was dried under a nitrogen stream and reconstituted to 200 µL in 95:5 water/acetonitrile.

Results & Discussion: A calibration curve containing a panel of 10 drugs, including xylazine, at concentrations ranging from 1 to 1000 ng/mL, was used for the quantitation of unknown whole blood samples. The lower limit of quantitation (LLOQ) was required to have an accuracy within ± 20% and a signal-to-noise ratio (S/N) greater than 10. For all 10 compounds, the LLOQ was successfully established at 1 ng/mL, which is sufficient for forensic screening methods.

Conclusion: In our screening process, a total of seven whole blood samples known to contain fentanyl were analyzed. Notably, two of these samples contained xylazine at concentrations above the LLOQ, measuring 7.4 ng/mL and 8.3 ng/mL. Additionally, three samples exhibited detectable levels of xylazine but were found to be below the LLOQ. It is worth highlighting that none of the seven samples contained solely fentanyl but exhibited the presence of 2 to 3 additional drugs and/or metabolites from the panel. This underscores the complexity and potential poly-drug use associated with the source of these samples, demonstrating the importance of comprehensive screening and quantitation techniques in such analytical contexts. Given that the data was acquired through a non-targeted SWATH DIA approach, the processing method developed in this study for the targeted screening of specific compounds can be readily adapted to identify unknown compounds.

Multi-target analysis of 174 synthetic cannabinoids in oral fluid using LC-MS-MS to monitor recent exposure and application to forensic cases

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Background & Aims: There has been considerable scientific curiosity surrounding the exploration of oral fluid as a potential specimen for drug testing. Oral fluid is an excellent biological matrix for clinical and forensic drug testing. It exhibits the ability to detect parent drugs, offering promising potential for noninvasive detection of relatively recent drug use. Furthermore, oral fluid demonstrates a stronger correlation with blood concentrations compared to urine. Synthetic cannabinoids (SCs) are the widest and most diffused class of new psychoactive substances, capable of producing psychoactive effects such as excitement, anxiety, and cognitive impairment. The rapidly evolving and intricate structure of synthetic cannabinoids (SCs) poses considerable challenges in their monitoring. Accordingly, multi-target methods that cover a wide range of SCs are preferred. The aim of this study is to develop and validate a multi-target ultra-high-performance liquid chromatography-mass spectrometry (UHPLC-MS/MS) method for the simultaneous determination of 174 SCs and related metabolites in oral fluid and to apply the method to authentic cases.

Methods: A 100 µL oral fluid sample was transferred to a 15 mL plastic tube, followed by the addition of 500 µL of phosphate buffer (pH=3) containing the internal standard. Extraction was performed using 2 mL of a n-hexane: ethyl acetate mixture (1:9, v/v), followed by vortex mixing and centrifugation. The supernatant was evaporated at 40°C and then dissolved in 100 µL of acetonitrile. The solution was centrifuged for 3 min at 9700 × g, and 5 µL was injected into the LC-MS/MS. Chromatography separation was achieved using a Waters Acquity UPLC HSS T3 column (100 mm × 2.1 mm, 1.8 µm). The mobile phase A and B was 20 mmol/L ammonium acetate and 0.1% formic acid in water and acetonitrile, respectively. The gradient elution was programmed as follows: 0–1 min 30% B, 1–3 min to 38% B, 3–4 min to 50% B, 4–6 min to 54% B, 6–6.5 min to 60% B, 6.5–9 min to 65% B, 9–9.5 min to 80% B, 9.5–10 min to 98% B; the column was then flushed with 98% B for 3 min. The total run time was 14 min. The mass spectrometer was operated with positive electrospray ionization and multiple reaction monitoring (MRM). The validated LC-MS/MS method was successfully applied to authentic cases, and the concentrations of synthetic cannabinoids in oral fluid were provided.

Results & Discussion: In this study, a baseline separation of most isomers was successfully achieved. The method demonstrated its capability in quantifying 160 SCs and related metabolites in oral fluid. The limits of detection and limits of quantification (LOQ) in oral fluid were in the range of 0.01–5 ng/mL and 0.02-10 ng/mL, respectively, with the majority of LOQs (88%) falling below or equal to 2 ng/mL. All substances exhibited goog linearity from their respective LOQs up to 100 ng/mL. This method exhibited acceptably precision and accuracy, with precision varying from 0.01% to 11.93% and accuracy from 86.29% to 109.63%. Notably, 30 SCs, including 4F-ABUTINACA, APINACA(AKB-48), 5-fluoro MPP-PICA, isobutyl 1-pentyl-1H-indazole-3-carboxylate, and MDMB-CHMCZCA, exhibited instability during long-term storage at −20°C for 30 days. Additionally, 5-fluoro MPP-PICA and isobutyl 1-pentyl-1H-indazole-3-carboxylate were found to be unstable after undergoing three freeze–thaw cycles. Therefore, it is advisable to store samples at −20°C, but it is crucial to limit the storage duration to a short period and refrain from repeated freeze-thaw cycles. The validated method was successfully applied to 11 authentic oral fluid samples from routine cases at the Academy of Forensic Science. MDMB-4en-PINACA and ADB-BUTINACA were detected in three of these cases. The concentrations of MDMB-4en-PINACA in oral fluid ranged from 0.02 to 3.8 ng/mL (n=3), while ADB-BUTINACA concentrations varied from 3.1 to 107.2 ng/mL (n=2). Additionally, the metabolite MDMB-4en-PINACA butanoic acid was detected in one case at a concentration of 5.6 ng/mL. It demonstrates the practical application of the established LC-MS/MS method for SCs in oral fluid.

Conclusion: A UHPLC-MS/MS method to detect and quantify more than 160 SCs and their metabolites in oral fluid in a single analysis with a good performance in the validation was developed in this study. The method is convenient and rapid, covers a wide range of substances, and exhibits high sensitivity. The applicability of the method was demonstrated by the analysis of authentic oral fluid samples. After validation the method has been applied to authentic cases and accumulated the concentration data of MDMB-4en-PINACA and ADB-BUTINACA in oral fluid of abusers.

Identification of compounds in products claiming to contain tetrahydrocannabinol analogs that have been distributed in Japan since 2023

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Background & Aims: Since around 2021, products claiming to contain Δ9-tetrahydrocannabinol (Δ9-THC) analogs have been distributed over the Internet and in shops on the street. Among these THC analogs are compounds with different lengths of the side chain at the C3 position of Δ9-THC and Δ8-THC such as tetrahydrocannabihexol (THCH), reduction reaction products of Δ9-THC and Δ8-THC such as hexahydrocannabinol (HHC), and compounds with acetylated hydroxyl groups at the C1 position of Δ9-THC and Δ8-THC such as THCO (Δ9-THC-O-acetate or Δ8-THC-O-acetate).

Putative THC-analog-containing products, including liquid cartridges for electronic cigarettes, herbal, gummy, and cookie products, are labeled with the name of the their contained THC analog. However, the actual components of these products distributed are not always known. In this study, we identified the compounds in products that claiming to contain THC analogs.

Methods: Seven e-cigarette cartridge products (A–F) and one herbal product G were obtained in Japan between November 2023 and March 2024. One-milligram samples of 1 mg of each product were extracted with 1 mL of acetonitrile under ultrasonication for 10 min. The resulting solutions were used for GC–MS and LC–MS measurements. After isolating and purifying unknown components from the product, structural analysis was performed by measuring 1H, 13C NMR and various two-dimensional NMR (COSY, HMQC, HMBC, and NOESY).

Results & Discussion: Δ9-THC-O-propionate and Δ8-THC-O-propionate, having a propionylated hydroxyl group at the C1 position of Δ9-THC and Δ8-THC, respectively, were detected in Product A, which purportedly contains "CBP". Δ9-THCM and Δ8-THCM, having a methylated hydroxyl group at the C1 position of Δ9-THC and Δ8-THC, respectively, were detected in Product B and Product C, respectively, which purportedly contain "THCM". 9(R)-HHCPM, in which the hydroxyl group at the C1 position of hexahydrocannabiphorol (HHCP) is a methylated and the alkyl side chain at C3 position of HHC is a heptyl group instead of a pentyl group, was detected in product D, which purportedly contains "HHCPM", The presence of 9(S)-HHCPM as a trace component was also suggested in Product D. GC-MS detected an apparent peak of [M]+ with m/z = 332 in the mass spectrum of Product E, which purportedly contains "PHC". As a result of structural analysis by NMR, it was determined to be 10(S)-hydroxy-9(R)-HHC, which has a hydroxyl group at the C10 position of HHC. Analysis of Product F, which claims to contain "HHCB", yeilded 9(R)-HHCB, dihydro-iso-THCB, and epossibly 9(S)-HHCB as a trace component. HHCB is a compound that differs in the length of the alkyl side chain of HHC. Dihydro-iso-THCB is a reduction product of Δ4(8)-iso-THCB and Δ8-iso-THCB, the reported byproducts of Δ9-THCB or Δ8-THCB synthesis via cyclization of CBDB. 5-butylbenzene-1,3-diol, considered as a synthetic raw material, was also detected in Product F. The compound Δ9-THCH-O-aceate, which has an acetylated C1 position at the hydroxyl group of Δ9-THCH, was detected in product G, which purportedly contains "THCHO".

This study reports the first detection of THC analogs having methylated and propionylated hydroxyl groups at C1 position of THC in distributed products. Reports have shown that Δ9-THCM induces catalepsy in mice and that THCO possibly expresses its activity through metabolism and deacylation to Δ8-THC or Δ9-THC in the body. Therefore, Δ9-THC-O-propionate and Δ8-THC-O-propionate might be similarly deacylated to express their activity in the body.

Conclusion: The THC analogs identified in Products A-G have not been reported or have been reported only in trace amounts in nature. In addition, some THC analogs in the products were accompanied by compounds that are probably synthetic byproducts or synthetic raw materials. Therefore, the THC analogs detected in this study are considered as synthetic. In addition, the newly detected THC analogs are a pottential health hazards if abused In the future, there are concerns about the distribution of products containing new THC analogs. Continuous provision monitoring is important for addressing any future concerns about the distribution of products containing new THC analogs.

NPS P-46

Dried blood spot (DBS) analysis of synthetic cathinones by different liquid chromatography–mass spectrometry techniques and interlaboratory validation for application in forensic toxicology

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Background & Aims: The identification of New Psychoactive Substances in biological samples represents a key factor for forensic and clinical toxicologists in diagnosing intoxications, addictions and disorders caused by the use of these substances and in assessing their prevalence in the community. The use of micro-extraction techniques

for sample pre-treatment, which allow the use of small amounts of sample and solvent, is becoming increasingly widespread with the aim of making analytical chemistry more environmentally friendly. Dried Blood Spot (DBS) is a minimally invasive microsampling method based on collecting blood spots on blotting paper and drying them.

Once dried, DBS samples do not require refrigeration and can be easily stored and shipped without special precautions.

The main objective of the present work is focused on the evaluation of DBS for the detection of twenty synthetic cathinones in blood samples, including from postmortem caseworks, the interlaboratory validation and the evaluation of the stability of cathinones in DBS, also studying authentic forensic caseworks.

Methods: For lab1, aliquots of 90 µL of blood were pipetted on the DBS cartridges (QIAGEN QIAcard WHAWB100014 Bloodstain cartridges by Merk, Milan, Italy) in three different 30µL spots. After deposition, the blood spots were allowed to dry for 2 hours at room temperature. Once dried, the blood spots were carefully cut out with scissors; each set of three spots was put in a glass tube with 500 µL of methanol/0.1% formic acid, added with the internal standards mixture and extracted in an ultrasonic bath. The supernatant was collected in another tube, evaporated to dryness under a nitrogen stream at room temperature, reconstituted in 100 µL of water with 0.1% formic acid and 10 µL of the sample were injected directly into the UHPLC-MS/MS system. The UHPLC-MS/MS, a Waters Acquity system, was equipped with a C18 column and analyte separation was performed by reversed-phase chromatography with gradient elution.

Mass acquisition was performed on a Xevo TQ-S micro triple quadrupole mass spectrometer in MRM mode. At least two transitions were selected for the identification of each substance.

For lab 2, aliquots of 85 µL of blood were pipetted on the DBS cartridges (Whatman 903® protein saver cartridges by Merk, Milan, Italy) on different spots. 2 h after deposition spots were cut, extracted with phosphate buffer solution under sonication, and finally supernatant was purified with SPE. The SPE eluate was dried under a nitrogen stream, and reconstituted in 200 µL mobile phase; finally, 5 µL were injected in the LC-MS/MS system. LC-MS/MS analyses were performed with an Agilent 1100–1200 Series coupled with a 4000 QTRAP. Chromatographic separation was achieved with C18 column gradient reversed-phase elution whereas mass acquisition was performed in MRM positive mode.

Validation was performed by the two laboratories involved in the study by evaluating selectivity, LOD, LOQ, linearity range, intra-day and inter-day precision, accuracy, matrix effect, recovery and stability at 7, 14, 60 and 90 days after deposition on the DBS cartridge.

For authentic postmortem samples, further experiments were performed in LC-HRMS for metabolites identification. The results obtained for these samples, using lab 1 and lab 2 pretreatment and LC-MS/MS conditions, were compared.

Results & Discussion: Validation results obtained in both laboratories demonstrated the suitability of DBS as sample storage and pre-treatment technique, allowing good sensitivities, repeatability, matrix effect and %bias. Limits of detection LOD and quantification LOQ ranged between 0.3 and 1 ng/ml and between 0.5 and 10 ng/mL, respectively, for the analytes included in the study in both laboratories. Stability studies demonstrated the high percent of degradation of cathinones, depending on their molecular structure, not attributable to DBS. Higher degradation rates were observed for cathinone itself and secondary amine cathinones, with a high percentage of decrease even 7 days after deposition, whereas methylenedioxy-substituted cathinones were more stable. The analysis of forensic caseworks containing α-PHP and MDPHP by DBS allowed their identification and quantification and the detection of various metabolites in postmortem blood samples. α-PHP degraded over time, while MDPHP concentration did not show significant differences in three months, probably due to the presence of the methylenedioxy moiety.

Conclusion: The obtained results demonstrate DBS to be an efficient technique for the analysis of cathinones, and that quantitative results for these analytes should be carefully interpreted due to their possible degradation. The evaluation of DBS by two different laboratories with different analytical techniques demonstrated the suitability of DBS for analysis in different settings, and the good agreement of results are very encouraging for large-scale application of the method by forensic and clinical toxicology laboratories

Metabolic profiling of the semi-synthetic cannabinoid hexahydrocannabihexol (HHCH)

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NPS P-47

Background & Aims: Hexahydrocannabinol (HHC) is a semi-synthetic cannabinoid, derived from cannabidiol (CBD), and emerged on the recreational drug market in 2021. As with any other new psychoactive substances (NPS), new derivatives of HHC have since appeared. One of them is hexahydrocannabihexol (HHCH) and has the hexyl group in place of the pentyl group of HHC. In Japan, several people have fallen ill and been sent to hospital after consuming gummies containing HHCH since September 2023, leading to the scheduling of HHCH as a "designated substance". However, little is known about the cannabinoid, including its metabolic pathways. The aim of this study was to identify the primary phase I metabolites of HHCH generated upon incubation with human liver microsomes.

Methods: HHCH was incubated with human liver microsomes for 1 h. After quenching the reaction with ice-cold methanol, the supernatant was analyzed by liquid chromatography–high-resolution mass spectrometry. Analytes were separated on a C18 column in gradient mode with the mobile phases of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. The mass spectrometric data were obtained in positive ion mode using data-dependent MS/MS mode.

Results & Discussion: In the employed conditions, thirteen metabolites were detected, predominantly formed through hydroxylation, ketone formation, and combinations thereof. The metabolite with the largest chromatographic peak area was derived from di-hydroxylation and ketone formation, while the second and third most abundant metabolites were mono-hydroxylated at the methylcyclohexyl moiety. Notably, among the thirteen metabolites, twelve were mono-hydroxylated at the methylcyclohexyl moiety, either with or without additional modifications elsewhere, indicating that mono-hydroxylation at this site is the primary metabolic pathway for HHCH.

Conclusion: The major metabolic pathway(s) of HHCH were found to be mono-hydroxylation of the methylcyclohexyl moiety. Thus, the two metabolites mono-hydroxylated at this moiety could serve as valuable markers for proving HHCH consumption.

NPS P-48

Creative cooking or sloppy housekeeping: a challenging judicial case in distinguishing intentional mixing and on-scene cross-contamination

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Background & Aims: Forensic laboratories are increasingly confronted with new psychoactive substances (NPS) that are readily available for purchase from a highly transient market. Consequently, routine analytical methods are put under pressure by the difficulties associated with the detection and identification of these novel and rapidly changing compounds. We present our recent experience with the analysis of a wide variety of powders, tablets, papers, plant materials and liquids, all recovered from the same location in 2023. In Belgium, a generic legislation prohibits various classes of psychotropic substances based on their respective common chemical structure since 2017. Our involvement in this judicial case encompassed the identification of the compounds in the seized items, and the determination of whether the Royal Decree of 2017 applies.

Methods: The lot of seized substances contained 14 powders, 12 types of tablets, 4 liquids, 2 samples of plant material, 1 type of capsule and 1 type of blotters. After visual inspection and photographical documentation of all materials, investigation of all powders, tablets and capsules was carried out by dissolving an accurately weighed portion of the substance in methanol, diluting and analysing using liquid chromatography coupled to diode array detection (LC-DAD) and gas chromatography coupled to mass spectrometry (GC-MS). All liquids were diluted in methanol and injected on GC-MS. One of the blotters was extracted with methanol, diluted and analysed with LC-DAD. The plant material was ultrasonically extracted in methanol, diluted and injected on GC-MS. Substances that could not be identified in the abovementioned way were more extensively investigated using LC coupled to quadrupole-time-of-flight mass spectrometry (LC-QTOF-MS).

Results & Discussion: The lot of seized substances contained a diverse array of items. Some of the most notable items were: tablets bearing the imprint "FANAX", a liquid labeled "Alegria Supernova", a bag with bright pink powder, multiple round tablets in various colors with the imprint "LL", and liquids labeled "Purple Mandala" or "Green Mandala".

Initially, extracts of the materials were injected on the LC-DAD and screened using an in-house library containing retention times and UV spectra. For seven items, a partial or complete identification of the substances, including "classical" drugs of abuse, was achieved. Furthermore, extracts were analysed using GC-MS and screened using multiple commercial libraries, resulting in 27 tentative identifications. The majority of the items showed peaks in the chromatograms that could not be unequivocally elucidated using LC-DAD and GC-MS, and needed LC-QTOF-MS analysis for identification and/or confirmation.

NPS were found in 28 out of 34 seized items. These designer compounds belonged to different classes, such as synthetic cathinones, phenylethylamines, benzodiazepines, ketamine derivatives and tryptamines. Five out of 34 identified NPS were not subject to the Belgian psychotropic substances legislation of 2017. Compounds are reported when the confidence level of identification is L3 or higher, according to Schymanski et al. Given the lack of analytical standards for all NPS and the caution required in reporting identification of these substances for legal consequences, the combination of several analytical techniques is important. Even so, we were unable to distinguish between positional isomers of some of the identified compounds, e.g. α-PHP or α-PiHP. In several items, combinations of NPS with "classical" drugs of abuse were detected.

An investigation of vendors' websites showed discrepancies in descriptions of the same product sold by different shops. In addition, we identified different or additional compounds compared to the claims of the vendors. For example, an online search for "Alegria Supernova" showed two vendors who described the content of the product as a mix of 2-MMC, 5-MAPB, 1P-LSD and 4-HO-MET. A third vendor mentioned 5-MAPB, 1P-LSD, 4-HO-MET and 3-CMC. However, the composition we reported, is: x-FMA, x-MAPB, x-APB, x-AcO-DET and x-HO-MET. While some advertised compounds are missing, we found extra products.

In addition to the identification of multiple NPS, we found remarkable, uncommon combinations in several tablets. In three tablets, a combination of a designer benzodiazepine (flubrotizolam or desalkylflurazepam) and the cathinone derivative α-P(i)HP was found, presenting a mixture of two chemicals with antagonistic effects. Other tablets contained a combination of a psychedelic phenylethylamine (2C-B derivatives) and a ketamine derivative (x-FDCK),. Since the batch contained powders that were respectively identified as α-P(i)HP and x-FDCK, a risk for cross-contamination during synthesis of these chemicals exists.

It is unclear whether the vendor intended to deliberately mislead users or the mixtures resulted from accidental contamination. It does raise the concern that (over)dosing with these substances containing compounds with different and even antagonistic modes of action can cause adverse reactions, also when given emergency care. Nevertheless, the seized items were in close contact, at the scene as well as during collection and storage by police services. As it is difficult and likely even impossible to trace the origin of a detected compound during analysis, the importance and difficulty of proper collection is illustrated in this complex case that involved such a wide variety of items.

Conclusion: This intricate case demonstrates that compounds of diverse nature can be found in one lot of seized items, implying that one vendor sells a wide array of substances, some also evading a national ban based on generic chemical structure. Discrepancies were noted between different vendors' web shops as well as between the advertised contents and our analytical results. In addition, the presence of substances with antagonistic effects and/or different properties in a single sample, complicates detection and raises concern for the health of users.

A fatal intoxication involving troparil, pagoclone and bromazolam

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Background & Aims: Troparil is a central nervous system stimulant and phenyltropane-based dopamine reuptake inhibitor, derived from methylecgonidine. It is more potent than cocaine as a dopamine reuptake inhibitor but is less potent as a serotonin reuptake inhibitor. Pagoclone is an anxiolytic agent from the cyclopyrrolone family, with similar effects to zopiclone. The aim is to report a fatal intoxication involving consumption of troparil and pagoclone with the results of the analysis of multiple matrices and paraphernalia.

Methods: A 29 year-old man was found dead in the bathroom of a hotel, after being missing for 24 hours. The cadaver was in an advanced stage of decomposition. Biological samples (blood, vitreous humor, urine, and gastric content) and the paraphernalia from the room (a plastic bag with yellowish white powder and 15 pills (10 green and 5 purple) were received in our lab. The composition of the yellowish white powder was investigated by HPLC-DAD and GC-MS. We consider of interest to carry out the structural elucidation of the compound identified as troparil to be able to use it as analytical standard. This was carried out in collaboration with the Research Technology an Innovation Center of Seville University (CITCUS) by NMR, HPLC-DAD, GC-MS. and UHPLC-QTOF. The pills composition was elucidated by GC-MS and UPLC-MS-ORBITRAP applying the Compound Discover program, followed by structural elucidation. Ethanol was determined in blood sample using the standardized INTCF method (GC-FID-HS). Biological samples were submitted to general unknown screening using enzyme immunoassay (CEDIA) and analysis by HPLC-DAD, GC-MS, UPLC-MS-ORBITRAP and GC-MS/MS after solid phase extraction. Unknown compounds were identified by UPLC-MS-ORBITRAP applying the Compound Discoverer program, followed by structural elucidation. The presence of carboxy-THC in the urine sample was investigated by GC-MS-MS.

Results & Discussion: The yellowish white powder contained troparil and carboxi-troparil (74:26)(m:m) and the pills contained bromazolam (green pills) and pagoclone (purple pills). Immunological screening of urine sample was positive for cannabinoids. Blood and vitreous humor samples gave negative results for ethanol and positive results for troparil, carboxy-troparil, bromazolam and pagoclone metabolites. A urine sample was positive for troparil, carboxi-troparil, pagoclone metabolites, carboxy-THC, and bromazolam and metabolites. The presence of troparil, and bromazolam and metabolites was detected in the gastric contents.

Conclusion: In the present case, the circumstances of death and toxicological results are consistent with the possible implication of two new psychoactive substances (NPS), troparil and pagoclone, in death. This case also reveals the importance of the paraphernalia to identify the substances consumed, especially when NPS are involved. To our knowledge, this is the first study describing troparil and pagoclone fatal intoxication.

NPS P-50

Metabolite profiling of 25I-NBOH in human liver microsomes using UPLC-Q-TOF-MS

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Background & Aims: N-(2-Hydroxybenzyl)-2-(4-iodo-2,5-dimethoxyphenyl) ethylamine (25I-NBOH) is a novel psychoactive substance, and its metabolism in vivo was not well understood. In this study, Ultra-high-performance liquid chromatography coupled with time of flight tandem mass spectrometry (UPLC-Q-TOF-MS/MS) was employed to identify and characterize the metabolites of 25I-NBOH. The acquired data were processed using Compound Discoverer software. A total of 11 metabolites were identified and characterized in human liver microsomes. Among these metabolites, M7, M9, and M10 were determined to be the most abundant.

Methods: Adding 200 ng of 25I-NBOH standard in the incubation solution after incubating with human liver microsomes , incubated at 37°C for 2 hours. Subsequently, the corresponding enzyme system (UGTs) for phase II metabolism was added to incubation system , incubated at 37°C for additional 30 minutes. The reaction system was terminated by the addition of 200 μL of cold acetonitrile. For analysis, liquid chromatography system equipped with a Luna Omega Polar C18 column (2.1mm × 100 mm, 1.6 μm), eluted with a gradient of 0.1% formic acid-water (A) and methanol (B) for 15 minutes. The metabolites and their biotransformation pathways were detected using ultra-performance liquid chromatography time-of-flight tandem mass spectrometry (UPLC-Q-TOF-MS/MS). To ensure the reliability of the results, three control groups were established:, group 1 (without addition of liver microsomes), group 2 (without addition of NADPH regeneration system solution), and control (without addition of 25I-NBOH).

Adding 200 ng of 25I-NBOH standard in the incubation solution after incubating with human liver microsomes , incubated at 37°C for 2 hours. Subsequently, the corresponding enzyme system (UGTs) for phase II metabolism was added to incubation system , incubated at 37°C for additional 30 minutes. The reaction system was terminated by the addition of 200 μL of cold acetonitrile. For analysis, liquid chromatography system equipped with a Luna Omega Polar C18 column (2.1mm × 100 mm, 1.6 μm), eluted with a gradient of 0.1% formic acid-water (A) and methanol (B) for 15 minutes. The metabolites and their biotransformation pathways were detected using ultra-performance liquid chromatography time-of-flight tandem mass spectrometry (UPLC-Q-TOF-MS/MS). To ensure the reliability of the results, three control groups were established:, group 1 (without addition of liver microsomes), group 2 (without addition of NADPH regeneration system solution), and control (without addition of 25I-NBOH).

Results & Discussion: By comparing the mass spectrum of the experimental group with those of the blank group and considering the fragment ion information of 25I-NBOH and its fragmentation pattern, we analyzed the potential metabolites using Metabolitepoilt 2.0 software based on information such as accurate mass number, isotopic distribution, and secondary mass spectrum of characteristic fragment ions. A total of 10 phase I metabolites and 1 phase II metabolite were identified from the total ion current graph of the sample. The main metabolic pathways observed included O-demethylation, hydroxylation, oxidation to ketones, and conjugation with glucuronic acid.

Conclusion: From the chromatographic peak areas of metabolites revealed that M7 (C17H20INO4), M8 (C17H21NO4), and M10 (C17H18INO4) exhibit the largest peak areas. However, due to the structural similarities between 25I-NBOH and 25I-NBOMe, M7 can also be produced from 25I-NBOMe. To avoid false positive results, M7 cannot be used alone as a metabolic marker. but needs to be combined with metabolites such as M8 and M10 for judgment/ It necessitates conjunction with other metabolites such as M8 and M10 for accurate judgment. The results of this experiment can provide a reference for studying the metabolic pathway of 25I-NBOH in humans.

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Identification of isomers and byproducts of the hexahydrocannabiphorol (HHCP) synthesis

Willi Schirmer[1,2], Ilche Gjuroski[2], Martina Vermathen[2], Julien Furrer[2], Stefan Schürch[2], Wolfgang Weinmann[1]
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Background & Aims: Since 2022 a new class of cannabimimetic compounds has appeared on the drug market. These compounds are isomers and derivatives of Δ9-tetrahydrocannabinol (THC) but were not regulated in the Narcotics Act. As these substances can be produced from cannabidiol (CBD) from Cannabis, they are called semi-synthetic cannabinoids. The first semi-synthetic cannabinoid to be legally sold as a marijuana alternative was hexahydrocannabinol (HHC), which was banned in Switzerland in March 2023.

New semi-synthetic cannabinoids such as hexahydrocannabiphorol (HHCP) have then emerged on the Swiss grey market to circumvent the law. These newer semi-synthetic cannabinoids differ in their alkyl chain length from natural cannabinoids such as THC or CBD. The sellers of these products claim that they are synthesized from natural CBD as a raw material, although there is no realistic chemical conversion to extend the alkyl chain into CBD.

The aim of this work was to get an insight into the source and synthetic procedures used to synthesize semi-synthetic cannabinoids which differ from the abundant cannabinoids THC and CBD in their alkyl chain length.

In October 2023, Switzerland introduced a class wide ban on semi-synthetic cannabinoids, which also includes the newer semi-synthetic cannabinoids such as HHCP.

Methods: A commercially available HHCP sample at the time was analyzed by GC-MS. In addition to the declared compounds 9S-HHCP and 9R-HHCP many other undeclared and unknown compounds were detected. The molecular ions and fragment ions of the unknown substances were similar to these of the declared HHCP epimers.

Six compounds were isolated by column chromatography on silica gel using mixtures of n-hexane and ethyl acetate as eluents. From these isolated substances only 9R-HHCP was a known compound. The structures of the other compounds were elucidated by GC-MS and various NMR experiments (1H-NMR, 13C-NMR, 1H/1H-COSY, 1H/13C-HSQC, 1H/13C-HMBC and NOESY).

The isolated compounds were derivatized with R- and S- Moshers acid chloride and measured with GC-MS to check their enantiopurity. A fraction which contained high molecular impurities was derivatized with MSTFA and analyzed with GC-MS to check for free reactive groups (phenols and enols).

Results & Discussion: Six compounds were isolated from the sample, four phenols (iso-HHCP, cis-(9R)-HHCP, 9R-HHCP, ortho-(9R)-HHCP) and two ketones which are presumably intermediates to cis-ortho-(9R)-HHCP and (9R)-HHCP). From the phenols only iso-HHCP was present as a scalemic mixture, the other phenols were enantiopure, proven by GC-MS analysis with their Mosher esters.

The fraction containing high molecular mass impurities consists of bisalkylated products, which appear during the synthesis of semi-synthetic cannabinoids. Characteristic fragments of the native and MSTFA-derivatized impurities were present in their respective mass spectra.

Conclusion: The impurity profile and the isolated compounds showed that this HHCP sample was made synthetically. It contained ketones which are presumably unreacted intermediates, unnatural stereo- and regioisomers and higher condensed molecules which are likely byproducts originating from the synthesis. Altoghether, our data prove that CBD or natural cannabis extracts were not used as starting materials for this sample as claimed by vendors of such cannabimimetic products.

Not much is known about the toxicological effects of semi-synthetic cannabinoids, and even less is known about the effects of unwanted by-products contained in these products due to poor quality control and lack of legal regulation.

Poster gallery – AM F-P-1 to P-43

16:00 - 16:30 Tuesday, 3rd September, 2024

AM F-P-01

Excretion of metabolites after oral ingestion of mitragynine

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Background & Aims: Kratom (Mitragyna speciosa) is widely used in Southeast Asia for its ethnomedicinal properties and has gained popularity in Western countries for recreational purposes. Mitragynine and 7-hydroxymitragynine are major alkaloids with affinity to opioid receptors. Since data on the potential risks and benefits of kratom use and abuse in humans is scarce, a phase I study was performed to evaluate the safety profile and acute effects as well as pharmacokinetic parameters of mitragynine. The present study aimed to elucidate the metabolism of pure mitragynine and to identify a suitable target compound to include in drug screening.

Methods: Human urine samples were collected before and after oral administration of mitragynine. For the detection of phase I metabolites, the samples were enzymatically cleaved and subjected to liquid-liquid extraction. Analysis was performed by LC-QToF MS in "All Ions" and "Auto-MS-MS" mode, as well as by LC-MSMS in precursor ion scan mode. Chromatographic peaks representing potential metabolites (candidates) were selected for further elucidation on the basis of extracted ion chromatograms of measured and predicted molecular masses and of characteristic fragments. Metabolites were then identified by interpretation of fragmentation data.

Results & Discussion: In a previous study (Philipp et al., J. Mass Spectrom. 2009), six phase I metabolites were detected in the urine of kratom users. Of these, 16-carboxymitragynine, 17-O-demethyl-16,17-dihydromitragynine and 9-O-demethylmitragynine, as well as unchanged mitragynine were confirmed, with 16-carboxymitragynine being identified as the most abundant metabolite. 9-O-demethyl-16-carboxymitragynine and 9,17-O-bisdemethyl-16,17-dihydromitragynine were not found. Several metabolites with a molecular mass indicating demethylation and hydroxylation were found, whose co-eluting characteristic fragment ions indicate their relation to mitragynine. Beyond the findings of Philipp et al., 7-hydroxymitragynine could be confirmed as metabolite using a reference standard. In addition, a number of further hydroxylated metabolites was found, which might represent isomers or rearrangement products (e.g. of the pseudoindoxyl type).

Conclusion: This study confirmed previously known mitragynine metabolites and provided some additional data. Apart from targeting the main kratom alkaloid mitragynine, the analysis of its major metabolite 16-carboxymitragynine (M+H: 385.2122, fragments m/z 174.0913, 212.1281, 224.1281) is suggested. However, the analytical results of urine from kratom users are much more complex, which most probably results from further indole-based alkaloids and their metabolites.

AM P-02

Rapid simultaneous detection of 24 drugs of abuse in DUID whole blood using LC-USI-MS/MS with on line solid phase extraction.

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Background & Aims: Driving under the influence of drugs of abuse cases poses significant challenges for law enforcement and forensic laboratories in identifying and quantifying these drugs in biological samples due to the complexity of the samples and the urgency of most of these cases. The use of short-time and inexpensive analytical methods has become an urgent need in the field, this presentation describe an online solid phase extraction analytical method which has been developed by using LC-USI-MS-MS for the detection and quantification of 24 drugs of abuse and their metabolites including Amphetamines, Cocaine, Benzodiazepines, Opioids and Pregabaline, with the application of the method in real DUI cases.

Methods: After a simple and rapid protein precipitation of 100 µL of whole blood sample using sulfosalicylic acid, all the 24 molecules were separated using a Waters® i-class Acquity ultra-performance liquid chromatography with an ACQUITY® UPLC BEH C18 1.7µm column after an online extraction method using an XBridge® C18 Direct Connect HP 10 µm column, and detected by WATERS® XEVO-TQS Micro tandem mass spectrometer using the new waters source of ionization UNISPARY® in MRM mode. Using a gradient of alkalinized water with 0.1% Ammonium Hydroxide and (Acetonitrile /Methanol/Acidified Water; 70:20:10, v/v/v) for the online extraction column, and

a gradient of Formic Acid/Methanol for the analytical column, At least two MRM transitions were monitored for each analyte, four deuterated internal standards were added to the samples at a concentration of 50 ng/mL.

Results & Discussion: The run time for the chromatographic method was 15 minutes resulting to the separation of all 24 analytes of interest including MDMA, COCAINE, MORPHINE, CLONAZEPAM, BROMAZEPAM and their principal metabolites. Quadratic calibration model was approved for all compounds, except for Morphine, Codeine, Dihydrocodeine, Pregabaline, and Midazolam which a linear calibration model was used, the concentrations of the lowest calibrator were 10 ng/mL to 2000 ng/mL. The coefficient of determination (R2) was above 0.998 with limits of quantification from 1 to 5 ng/mL. Linearity, accuracy, precision, repeatability and intermediate precision were within required limits.

Conclusion: A rapid, accurate and reliable novel qualitative and quantitative online solid phase extraction by LC-USI-MS/MS method was developed for the confirmatory analysis of 24 drugs of abuse and their metabolites in whole blood samples, the method has been used in routine analysis on 712 real cases of driving under the influence of drugs during the year of 2023 enabling the detection of all analytes of interest and producing a consistent results that confirmed what we obtained from chemiluminescence screening .

A comparison of approaches for source parameter optimisation strategies for multi-component LCMSMS methods – a journey from uninspired to super nerdy

AM P–03

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Background & Aims: The use of LCMSMS methods to monitor specific analytical targets is widespread in toxicology. A question that is often asked at the beginning of method development is, what is the best way to optimise the conditions of the mass spectrometer to meet the needs of sensitivity and performance of the assay?

Usually, the biases of the individual creating the method guide the procedure which is applied. One common approach is to use the built-in tools offered by vendor's software to perform semi-automated optimisation or to use what "has worked in the past" but are either of these approaches the best way?

In this work, several approaches to developing source conditions for a multi-analyte method (including a large range of medications and drugs of abuse) will be examined. These will include simple approaches such as the popular `guessing' or `borrowing from others' to more thorough methods implementing one factor at a time (OFAT) or sophisticated (read: complicated), combinatorial source method creation and fractional factorial design statistical directed experimental design approaches.

Methods: Using previously used compounds with optimised compound-specific parameters (Declustering Potential, Collision Energy), a new multi-analyte method was assembled using Scheduled MRM acquisition. For all source optimisation experiments, no modifications were made to the LC or compound-specific parameters. Several different source optimisation methods were applied to the method and the best conditions for each compound were examined to guide the choice of a final "best" method based on the peak area response of the analytes - specifically focusing on the poorest 10 analytes.

Results & Discussion: Simply applying previous method conditions to new methods was the fastest option available as no testing of different factors was performed. Using combinatorial parameter combinations was by far the most time-consuming method, however, it proved to be extremely thorough, providing a straightforward approach to selecting the best conditions for each analyte with the advantage of being relatively easy to set up. As an alternative approach, the compounds were simultaneously optimised for detection by fractional factorial design and face-centred composite design for optimal signal generation to achieve the best sensitivity in a fraction of the instrument time while achieving compatible final conditions as more extensive methods.

Conclusion: Each method for optimisation has its advantages and disadvantages. The method used will depend on laboratory-specific factors including the sensitivity of the instruments available, the operator skill plus the resources and time available to devote to method development. Importantly, the panel of analytes being examined needs to be carefully reviewed so that method development can focus on the critical few to ensure the overall goals of such methods are met with minimal time and effort being expended.

AM P-04

Determination of multiple class of drugs in urine and dried urine spots (DUS) and the evaluation of the stability over a 3-week period

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Background & Aims: The analysis of dried urine spot (DUS) guarantees an alternative simple urine sample storage that allows to obtain reliable analytical results even after a long period from collection time. However, the lack of validated methods and the limited volume of stored sample may affect the reliability of this analysis. The aims of this study were: a) to develop a target screening LC-MS/MS approach, based on parameters provided by previously published studies, for the detection of opioids (methadone, EDDP, oxycodone, tapentadol, tramadol, fentanyl, carfentanyl, furanyl fentanyl and ocfentanyl), and synthetic cathinones (MDPV and 3,4-MD-α-PHP), ketamine and norketamine in DUS collected from real case (including post-mortem and urines collected from people belonging to addiction services) ; b) to compare the results obtained with and without reference standards.

Methods: A sample of 50 µL of urine was deposited on a commercial card used for dried matrix spot analysis. The whole urine stain was manually cut and put in a glass tube, containing 1 mL phosphate buffer at pH 6 and four different deuterated internal standards at the concentration of 100 ng/mL. 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 75 µL mobile phase; finally, 5 µL was injected in the LC-MS/MS system. The analytes were separated through reverse phase chromatography on a C18 column and detected on a triple quadrupole operating in Multiple Reaction Monitoring (MRM) mode, in positive ionization, monitoring two transitions for each analyte. After validation of the analytical procedure, the method was applied to 20 urine samples.

Results & Discussion: The method is simple and relatively fast. The MRM transitions of the 12 monitored compounds were obtained from certified standards and the method was validated on all the substances of the study, which allowed to obtain a LOD range between 0.07 ng/mL (fentanyl) and 2.73 ng/mL (furanyl fentanyl). Subsequently, the stability of the compounds was evaluated throughout a period of 22 days, once a week, by the analysis of spiked samples at 10 ng/mL, 20 ng/mL, 50 ng/mL, 100 ng/mL, 250 ng/mL, 500 ng/mL, 1000 ng/mL for "classic" drugs and synthetic cathinones, and spiked samples at 1 ng/mL, 5 ng/mL, 10 ng/mL, 25 ng/mL, 50 ng/mL, 75 ng/mL, 100 ng/mL for fentanyl and its derivates. Three spiked samples for each concentration was prepared at the same time and stored at room temperature away from lights. Every seven days, each catridge was cut out and the solid phase extraction was performed. The remarkable stability of fentanyl, norketamine, tapentadol and tramadol, which present an almost unchanged concentrations of the analyte over the evaluated period, was demonstrated (maximum 15% in concentrations loss compared to the first analysis). On the contrary, the results of 3,4-MD-alpha-PHP, MDPV, ketamine and ocfentanyl, confirmed a relatively limited degradation, whether compared to data obtained for carfentanyl, methadone and oxycodone. Finally, a loss up to 50% of EDDP and furanyl fentanyl concentrations was observed after only two weeks. However, the method was able to qualitatively identify the presence of all the monitored substances within the monitored period.

Conclusion: A target method for the detection of 12 drugs of abuse in DUS has been successfully developed. Preliminary results on authentic positive cases suggested that DUS collection is of great interest and could be a good, simple, reliable, and complementary method of sample storage. The identification of all the substances included in the study was confirmed up to one month storage at room temperature. Stability represents the main issue that limits the reliability of quantitative results for most of the monitored compounds.

AM P-05

Evaluation of analytical validation parameters in blood alcohol concentration determination using the validaR application

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Background & Aims: To ensure quality in forensic laboratories, analytical validation of the methods used for toxicological analysis is essential, for example, in cases such as blood alcohol concentration determination, which is one of the most frequent assays in forensic laboratories. The availability of practical tools that help to perform the task of analytical validation of methods is a point highly valued by forensic toxicologists. Many statistical tools serve to perform analytical validation, we present the validaR application which is a tool for the validation of quantitative analytical methods created by the National Institute of Metrology of Colombia, available free on a page designed with the Shiny library of the R statistical programming language, this performing calculations automatically previous data incorporate. The objective was to evaluate the analytical validation parameters of the method for blood alcohol concentration determination using the validaR application. To ensure quality in forensic laboratories, analytical validation of the methods used for toxicological analysis is essential, for example, in cases such as blood

alcohol concentration determination, which is one of the most frequent assays in forensic laboratories. The availability of practical tools that help to perform the task of analytical validation of methods is a point highly valued by forensic toxicologists. Many statistical tools serve to perform analytical validation, we present the validaR application which is a tool for the validation of quantitative analytical methods created by the National Institute of Metrology of Colombia, available free on a page designed with the Shiny library of the R statistical programming language, this performing calculations automatically previous data incorporate. The objective was to evaluate the analytical validation parameters of the method for blood alcohol concentration determination using the validaR application.

Methods: The validaR v.1.0.3.3 application was used to evaluate the available validation parameters such as selectivity, precision, trueness, linearity, limit of detection, and robustness, for this we used as example method for blood alcohol concentration determination by gas chromatography with flame ionization detector coupled to headspace used by the Medico-Legal Service Laboratory; with calibrations ranging from 0.10 to 4.0 g/L for ethanol. The ANSI/ASB Standard Practices for Method Validation in Forensic Toxicology is used as guidelines, obtained data was income to the aplication for calculate parameters results. The review and detection of outliers of the data was performed using the BAC-Outliers v.1.0.2 application.

Results & Discussion: No outliers were detected in the data review; when using the validaR application in the blood alcohol concentration determination, the method proved to be linear within the range of calibrators with an r2 greater than 0.99, the detection limit was 0.03 g/L, the inter- and intra-round precision was less than 20% in the three concentration levels evaluated and the method of analysis is robust.

Conclusion: The evaluation of the available validation parameters of the method for blood alcohol concentration determination with the validaR application was performed satisfactorily, making this application a good tool, since it provides valuable information to ensure the quality of toxicological analysis.

The application could be used as a model or reference for future tools focused on analytical validation in the area of forensic toxicology.

AM P-06

Enhanced liquid phase microextraction: An approach for simultaneous detection of buprenorphine and norbuprenorphine in commercial plasma

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Background & Aims: Opioid use disorder(OUD) is associated with a lifelong struggle to control drug consumption, it is a chronic, recurrent disease. Although medications such as methadone, buprenorphine/naloxone, and naltrexone are highly effective in the treatment of opioid use disorder (OUD), a significant portion of patients experience cravings for the substance, which may lead them to continue illegal opioid use. This contributes to the observation that only 20 to 40% of individuals diagnosed with opioid use disorder are currently receiving treatment[1]. Buprenorphine is considered to have little respiratory side effects at therapeutic doses but still, there are several reports of buprenorphine-related deaths. Furthermore, its prescription formulations improve treatment accessibility, potentially contributing to the development of dependence[2]. Therefore, the accurate determination of buprenorphine in human plasma samples is crucial for effectively monitoring treatment efficacy and adherence. The present study aims to develop a green microextraction approach for the sensitive detection of buprenorphine and its primary metabolite in commercial plasma using HPLC-UV.

Methods: A novel HPLC-UV method utilizing a Kinetex F5 (Phenomenex, USA) analytical column (150 mm × 4.6 mm i.d., 5 µm) and a mobile phase flow rate of 0.75 mL/min was developed. The mobile phase comprised phosphate buffer (15 mM, pH 4) as mobile phase A and acetonitrile as mobile phase B. The gradient program initiated at 90% mobile phase A, with a subsequent linear increase in the proportion of mobile phase B until reaching 90% at 10 minutes. Detection was performed at a wavelength of 210 nm, with each sample injected at a volume of 10 µL.

Results & Discussion: The method was validated according to selectivity, linearity, LOD, LOQ, precision, and accuracy criteria specified in the International Council for Harmonisation guidelines. The method was found linear in the concentration range of 0.2-5 µg/mL for both analytes with correlation coefficient (R2) values greater than 0.999. Intra- and inter-day accuracy ranged from -2.58% to -9.91%, while precision for both analytes was below 5.55%.The limits of detection (LOD) and quantification (LOQ) for the method were established using signal-to-noise ratios. The LOD was found to be 0.05 µg/mL and 0.2 µg/mL for both compounds. The lower limit of quantification (LLOQ), defined as the lowest concentration of analyte with accuracy within 12% and precision <5.55%, was determined at 0.2 g/mL for both buprenorphine and norbuprenorphine.

Conclusion: This rapid extraction and analysis method offers a promising approach for simultaneously detecting buprenorphine and its main metabolite, customizing treatment and improving its effectiveness. This methodology can support monitoring treatment efficacy and compliance, especially for individuals at a higher risk of treatment failure.

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AM P-07

LC-MS/MS quantification of kratom alkaloids in human plasma

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Background & Aims: Mitragyna speciosa Korth, also known as Kratom, is a medicinal plant indigenous to South-East Asia that has a complex pharmacology including partial μ-opioid agonist, α-adrenergic, 5-HT1A, 5-HT2C & 5-HT7 serotonin, D2 dopamine agonist and A2A adenosine & κ-opioid antagonist. Most importantly, mitragynine does not activate the β-arrestin-2 pathway implicated in respiratory depression. In recent years, Kratom gained popularity in the United States for self-treatment of chronic pain, anxiety and opioid withdrawal and dependence; however, the pharmacokinetics/ pharmacodynamics and toxicokinetics/ toxicodynamics in humans remain poorly understood. We developed and validated a liquid chromatography- tandem mass spectrometry (LC-MS/MS) method for the simultaneous quantification of eleven Kratom alkaloids and metabolites in human plasma including mitragynine, 7-OH-mitragynine, 3-dehydro-mitragynine, mitragynine-pseudoindoxyl, speciociliatine, speciogynine, paynantheine, corynantheidine, mitraphylline, corynoxeine and isocorynoxeine.

Methods: For calibration standards and quality control samples, human plasma was fortified with alkaloids at varying concentrations within the working range of the respective compound. 200 µL sample was extracted using a simple one-step protein precipitation procedure. The extracts were analyzed using LC-MS/MS running in the positive multiple reaction monitoring (MRM) mode.

Results & Discussion: The assay was validated according to applicable Clinical Laboratory Standards Institute (CLSI) and United States Food and Drug Administration (FDA) guidelines for bioanalytical assays. The lower limits of quantification ranged from 0.5 to 1.0 ng/mL and the upper limit of quantification was 400 ng/mL for all analytes. Intra-day analytical accuracy and precision ranged from 102 to 113 % of the nominal concentrations and from 1.4 to 8.5% CV (coefficient of variance), respectively, and inter-day analytical accuracy and imprecision ranged from 98.4 to 113% of nominal and from 3.9 to 14.7% CV, respectively. Mean extraction efficiencies were 89.4 ± 6.7% for mitraphylline and 101 ± 11.1% for mitragynine-pseudoindoxyl and mean absolute matrix effect was -1.6 ± 1.6% for mitragynine-pseudoindoxyl and 61.9 ± 5.9% for corynoxine in plasma. All analytes were stable when stored up to 6 months at -80°C with concentrations within ±15.1% and after three freeze-thaw cycles, concentrations for all analytes were within ±10.9%. No carryover was detected. All analytes passed acceptance criteria for validation. The assay was used to analyze a subset of plasma samples collected as part of a double-blind, placebo-controlled clinical trial investigating the pharmacokinetics, safety and tolerability of Kratom in healthy participants. Capsules containing 500 mg of Mitragynine speciosa leaf powder including 6.92 mg of mitragynine, 0.71 mg of 3-dehydro-mitragynine, 0.85 mg speciogynine, 1.74 mg of speciociliatine, 0.15 mg of corynantheidine and 0.97 mg paynantheine, were administered. Participants were randomized in four dose groups: the low dose received 1 capsule, the mid dose-1 2 capsules, the mid dose-2 4 capsules and the high dose 8 capsules. Plasma samples for pharmacokinetic analysis were collected after a single dose administration and after 15-days repeated daily doses. Quantifiable concentrations of mitragynine, 7-OH-mitragynine, speciogynine, speciociliatine and paynantheine were detected in most samples for all condition tested while corynantheidine were not detectable in the low dose group and 3-dehydro-mitragynine was detected only in the high dose group.

Conclusion: In the present study, we developed and validated the first LC-MS/MS assay for the specific quantification of eleven Kratom alkaloids and metabolites in human plasma. Our aim is to use this assay to support clinical and observational studies on Kratom.

Comparative study of methadone quantification methods in plasma samples

AM P–08

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Background & Aims: The treatment of opioid dependence often involves the use of methadone, necessitating regular monitoring of plasma concentrations to ensure therapeutic efficacy and avoid adverse effects. This study aims to compare three analytical methods for methadone quantification in plasma samples from patients undergoing methadone maintenance treatment : immunoenzymatic assay, gas chromatography-mass spectrometry (GC/MS), and high-performance liquid chromatography with diode array detection (HPLC-DAD).

Methods: A total of 102 plasma samples from methadone-treated patients were utilized in this study. The plasma samples were stored at -20°C until analysis. The pretreatment step for the samples involved a simple dilution for the immunoenzymatic method, whereas a common liquid-liquid extraction using hexane/ether (70/30%) was employed for the samples analyzed by GC/MS and HPLC-DAD. Methadone quantification was performed using three different methods: an immunoenzymatic assay using the Randox technology device, GC/MS by Agilent, and HPLC-DAD by Perkin Elmer. Each method was evaluated based on sample volume, analysis time, calibration range, detection principle, and limit of detection. Correlation coefficients between the methods were calculated to assess their agreement.

Results & Discussion: The study revealed strong correlations between the analytical methods employed. The correlation coefficient between GC/MS and HPLC-DAD was 0.9669, indicating a high degree of agreement. Similarly, significant correlations were observed between GC/MS and the immunoenzymatic method (coefficient = 0.9079) as well as between the immunoenzymatic method and HPLC-DAD (coefficient = 0.9438). for the Bland-Altman test: the majority of points were between the limits of agreement, this indicates good agreement between the assay methods. These findings suggest consistent results across different analytical platforms. Notably, each method exhibited distinct advantages and limitations, such as differences in detection limits and calibration ranges. Despite these variances, the overall agreement between the methods underscores their reliability for methadone quantification in plasma samples.

Conclusion: This study demonstrates the feasibility and reliability of immunoenzymatic assay, GC/MS, and HPLC-DAD methods for quantifying methadone in plasma samples from patients undergoing methadone treatment. The strong correlations observed between the methods indicate their interchangeability in clinical practice. However, the choice of analytical method may depend on factors such as sensitivity, analysis time, and equipment availability. Further studies are warranted to optimize analytical protocols and validate their suitability for routine clinical use.

Miniaturized sample preparation techniques for organophosphorus compound analysis in blood: a preliminary recovery study

AM P–09

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Background & Aims: Poisonings involving pesticides, including organophosphorus (OP) compounds, present significant public health concerns worldwide. These compounds are commonly encountered in agricultural settings and industrial processes. Notably, pesticide poisonings often result in severe health consequences. Organophosphorus compounds, are among the pesticides frequently implicated in poisoning cases in Malaysia. The identification and quantification of these compounds in biological samples are crucial for toxicology investigations. However, traditional sample preparation methods for OP analysis in blood samples are often labor-intensive and time-consuming, highlighting the need for more efficient techniques.

The aim of this study is to explore miniaturized sample preparation techniques for the analysis of OP compounds in blood samples, focusing on enhancing the efficiency of the analyses. By optimizing sample preparation protocols, the study seeks to streamline the analytical workflow for OP compounds. This preliminary investigation aims to lay the groundwork for future research in developing novel sample preparation methods tailored to the specific requirements of forensic toxicology, ultimately contributing to more accurate and timely detection of OP exposure in poisoning cases.

Methods: The sample preparation procedure begins with the transfer of 1 mL of OP-fortified blood specimen into a microtube, followed by the addition of 0.4 mL of ethyl acetate. Afterward, the mixture undergoes vortex shaking for 2 minutes to ensure thorough extraction of the OP compounds. Subsequently, the microtube is centrifuged for 2 minutes to separate the organic layer containing the extracted compounds. Finally, the supernatant is carefully transferred to a gas chromatography (GC) vial for analysis using full scan gas chromatography-mass spectrometry (GC-MS) utilizing the Agilent J&W DB-5MS column, facilitating the identification of the OP compounds present in the sample. The GC oven program for this analysis begins with an initial temperature of 80°C, maintained for 2 minutes. Following this, the temperature ramps up at a rate of 25°C per minute until it reaches the final temperature of 300°C. The final temperature is held for a duration of 7 minutes. The total run time for this oven program is 17.8 minutes.

Results & Discussion: The recovery rates obtained for various OP compounds at a concentration of 1.0 ug/mL in the OP-fortified blood reveal important insights into the efficiency and reliability of the sample preparation method and subsequent analytical procedure. Recovery studies were conducted on triplicate samples. The recovery percentage for each analyte is calculated by comparing the concentration in the extracted sample to the expected theoretical concentration, which is the concentration of the analyte added to the sample prior to extraction (= 1.0 ug/mL). Among the compounds tested, ethoprophos, fenamiphos, fenchlorphos, coumaphos, and ethyl primiphos displayed excellent recovery rates ranging from 97% to 117%. These high recovery rates suggest robust extraction efficiency and reliable detection capabilities for these compounds, indicating the suitability of the analytical method for their quantification in blood samples.

Conversely, several compounds exhibited lower recovery rates, including terbufos, disulfoton, methyl primiphos, phenthoate, methyl tolclophos, and leptophos, with recovery rates ranging from 42% to 78%. The lower recovery rates observed for these compounds may be indicative of challenges in their extraction or analytical detection, potentially stemming from differences in their chemical properties, such as solubility, volatility, or stability. These findings underscore the importance of method optimization and validation to improve the recovery of compounds with lower efficiency and ensure the accuracy of forensic toxicology analyses.

Conclusion: The findings from this study highlight the critical importance of developing efficient and reliable sample preparation techniques for the analysis of OP compounds in blood samples. The high recovery rates observed for certain compounds, such as ethoprophos, fenamiphos, fenchlorphos, coumaphos, and ethyl primiphos, indicate robust extraction efficiency and reliable detection capabilities for these compounds. However, the lower recovery rates observed for other compounds, including terbufos, disulfoton, methyl primiphos, phenthoate, methyl tolclophos, and leptophos, highlight the need for further optimization and validation of sample preparation methods. The variability in recovery rates among the tested compounds underscores the complexity of forensic toxicology analyses and emphasizes the importance of considering individual compound characteristics and potential matrix effects. Overall, this study contributes valuable insights into the development of sample preparation techniques tailored to the specific requirements of forensic toxicology, ultimately aiming to improve the accuracy and reliability of detecting OP compound exposure in poisoning cases, thus enhancing public health outcomes and forensic investigations.

AM P-10

Determination and validation of anxiolytics, anticonvulsants, and illegal drugs in meconium by LC-MS/MS to indicate in-utero exposure

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Background & Aims: Drug and illicit substance abuse during pregnancy is a significant public health problem. This is because in utero substance exposure can affect the development of the newborn and therefore the child's long-term behavioral, cognitive and developmental abilities. There is little data on drug and illicit substance use during pregnancy. The most common methods used to detect drugs and illicit substance use during pregnancy are maternal interviews or surveys. In our country, according to our knowledge, no data has been found regarding the determination of drugs and illegal substance in meconium. In this study, we aimed to develop and validate an appropriate extraction method for anxiolytics (bromazepam, lorazepam, oxazepam, alprazolam, flunitrazepam, diazepam, nordiazepam, medazepam, flurazepam, chlordiazepoxide, moclobemide), anticonvulsants (clonazepam,7-aminoclonazepam, carbamazepine, oxcarbazepine, clobazam, nitrazepam, gabapentin, pregabalin), and illegal drugs in meconium by liquid chromatography /tandem mass spectrometry.

Methods: Meconium sample (250±10 mg) was weighed into a 16x100 mm glass tube, followed by the addition of 2 mL of methanol and homogenized by ultrasonication for 30 min until uniform, then centrifuged at 3500 rpm for 10 min. The supernatant was transfer to another tube.

A solid-phase cartridge (OASIS MCX 60 mg, 3 cc) was used for sample extraction. Firstly, an SPE cartridge was conditioned with 2 mL of methanol and 2 ml of deionized water. The sample was passed through the cartridge and washed with 2 mL of 2% formic acid in pure water and 2 mL methanol:water: formic acid (47.5:47.5:5). The cartridge was dried under vacuum for 15 min. Eluent was subsequently extracted with 2 mL of a mixture of dichlorometan:2-propanol: ammonium hydroxide (20:78:2, v/v). After the extract was dried under nitrogen, it was reconstituted in 250µL methanol. A sample of 20 µL was injected into the LC- MS/MS. The LC-MS/MS analysis was performed on a Shimadzu Nexera chromatography system equipped with an electrospray interface (ESI) in MRM mode. The chromatographic separation was performed with a pentafuorophenylpropyl (PFPP) column (Allure 50×2.150 mm i.d., 5 µm, Restek, Bellefonte, PA, USA) using a gradient binary with 10 mM ammonium formate (A) in ultrapure water and methanol (B). The run time is 20 min.

Results & Discussion: The method was validated in terms of limits of detection (LODs) (0.14–7.28 ng/g) and quantifcation (LOQs) (0.44–22.06 ng/g), extraction recovery (ER%) (65.0–110.0%), matrix effect (ME%) (12.2-99.9 %), linearity (r2> 0.99), intra- and interday precision (CV< 20%) and carryover. In this study, we compared two different extraction cartidge (HLB and MCX) in meconium. In many routine laboratories, a single extraction procodure is preferred to reduce laboratory costs and HLB cartridge is widely used. However, it was seen in our study that gabapentin and pregabalin were detected with HLB cartidge only in very high concentrations.

Conclusion: A simple, cost-effective, and accurate LC/MS–MS method has been developed and validated for anxiolytics, anticonvulsants and classic illegal drugs. It has been suggested that the MCX cartridge should be preferred in meconium for these drugs.

Development of a simultaneous quantitation method for 203 illicit drugs and metabolites in urine using LC-MS/MS

AM P-11

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Background & Aims: Drug abuse has become a significant concern worldwide, casting a shadow over national safety and welfare of society. The range of illicit drugs and drug markets is continuously growing. However, the simultaneous detection method for a broad range of drugs of abuse (DOAs) in biofluids faces limitations due to the presence of matrix effects and the diverse physicochemical properties of drugs. In this study, we aimed to develop simultaneous analysis methods for quantifying 203 DOAs and metabolites in 100 µL of urine using LC-MS/MS. The developed method presented two efficient sample preparation methods: the dilution method involving enzyme hydrolysis and QuEChERS. Furthermore, a separate LC-MS/MS method was developed to detect three drugs for better sensitivity.

Methods: We targeted six classes, including a total of 203 illicit drugs: amphetamines, opiates, cathinones, phencyclidines, synthetic cannabinoids, cocaine, and their metabolites. The selection of recently abused drugs as target substances was based on a comprehensive review of literature from both domestic and international sources. The effectiveness of two sample preparation methods was compared in urine samples: one involved dilution with enzyme hydrolysis, while the other utilized QuEChERS with Acetonitrile (ACN). For the LC-MS/MS method, a C18 column with a 16-minute gradient program was used to separate the target compounds. Mobile phase A consisted of 10 mM ammonium formate in water (0.1% formic acid), while mobile phase B consisted of 10 mM ammonium formate in methanol (0.1% formic acid). A multiple reaction monitoring (MRM) mode with ESI source was adapted and optimized for the simultaneous detection of all 203 target compounds. ESI was operated in positive/negative ion switching mode. Intra- and inter-laboratory validation were performed to assess the efficacy of the developed methods.

Results & Discussion: The method was validated based on the Standard Practices for Method Validation in Forensic Toxicology (ANSI/ASB Standard 036, 1st Ed. 2019) with the following parameters: specificity, sensitivity, matrix effect, linearity, intra- and inter-day accuracy, and precision. As a result, the developed LC-MS/MS method demonstrated good linearity, with 98.5% of the targeted compounds (n=200) exhibiting coefficient of determinaton (R2) exceeding 0.99. The LOQ of 203 compounds ranged from 0.1 to 20.0 ng/mL. The intra- and inter-day precision was below 20% for all targeted compounds, and most targeted compounds (198 out of 203) showed accuracy ranging from 80 to 120% at 10 ng/mL . When comparing two sample preparation methods, dilution with enzyme hydrolysis and QuEChERS with acetonitrile (ACN), both showed good linearity and low matrix effect. A separate LC-MS/MS method was developed to detect GHB, GBL, and 1,4-butanediol for better sensitivity. The urine samples for the

detection of GHB, GBL, and 1,4-butanediol were prepared with the same hydrolysis process and diluted with water. The C18 column with a 13.5-minute LC gradient program was used. Mobile phases A and B consisted of 0.02% formic acid and methanol, respectively. ESI was operated in positive/negative ion switching mode. The developed simultaneous method targeting 203 compounds was successfully adapted for the 40 real urine samples.

Conclusion: The simultaneous analysis method for 203 illicit drugs with six classes, using LC-MS/MS, was developed and demonstrated its effectiveness in intra- and inter-laboratory validation. The developed method is considerably useful as it can simultaneously detect a variety of DOAs with various physicochemical properties only in 16 minutes by using 100 µL of urine. The technique could be adapted to meet the practical needs of forensic investigations, thereby improving its effectiveness and reliability.

AM P–12

A validated, efficient and robust LC-MS/MS method for quantitation of Δ9-tetrahydrocannabinol and its main metabolites, hexahydrocannabinol and cannabidiol in human plasma and blood

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Background & Aims: Highly increasing numbers of driving under the influence of drugs (DUID) cases have been observed all over Austria in recent years. Main explanations are extended police and medical resources as well as improved training of police officers involved in vehicle and driver inspections. The number of DUID cases analyzed at our institute, covering Western Austria, multiplied by a factor of about 10 in the last five years. Among these cases cannabis impairment was the predominant result. Moreover, with legalization of cannabis consumption in several countries such as Germany in 2024 an increasing number of cannabis positive cases are to be expected within all forensic fields.

The challenge of keeping pace with increasing sample numbers met in addition the challenge of newly emerging controlled cannabinoids such as hexahydrocannabinol (HHC) and the increasing occurrence of the widely promoted cannabidiol (CBD). First, the analytical capacity for analysis of the "established" cannabinoid Δ9-tetrahydrocannabinol (THC) and its main metabolites 11-OH-THC and THC-COOH at our institute had to be extended. Second, additional analytical procedures concerning HHC and CBD had to be developed. Our aim was therefore to establish an efficient and robust LC-MS/MS method based on the available (low-end) instrumentation enabling the simultaneous determination of THC, 11-OH-THC, THC-COOH, HHC and CBD.

Methods: Human plasma or blood samples (250 µl) were extracted by an acetonitrile protein precipitation step followed by solid phase extraction (Chromabond Drug II, Macherey-Nagel). Chromatographic separation was achieved on a reversed-phase C18 column (2.0 x 100 mm, 5 µm particle size, Eurosphere II, Knauer) within a run time of 15 min. Detection was performed on a 3200 QTRAP instrument (Sciex) in positive multiple reaction monitoring mode. Matrix matched six-point calibrations were generated in blank human plasma applying deuterated analogues as internal standards for all analytes except for HHC. The method was fully validated according to the GTFCh guidelines.

Results & Discussion: Linear ranges were 0.5-25 µg/l for THC, 11-OH-THC, HHC and CBD, and 2.0-100 µg/l for THC-COOH, respectively. Limits of detection and limits of quantification were 0.5 and 1.0 µg/l (THC, 11-OH-THC, HHC, CBD), and 2.0 and 4.0 µg/l (THC-COOH). Applicability of matrix matched plasma calibrations to human full blood samples was successfully demonstrated. Acceptance criteria for intra- and inter-batch accuracy (85-115 %) and precision (<15 %) were met for all compounds in human plasma and blood. Mean extraction efficiency was 74.1-79.7 % and 67.6-78.2 %; mean matrix effects ranged from 76.6-90.0 % and 81.8-110.8 % for plasma and blood samples, respectively. No carry over was observed and no interfering signals were detected for samples spiked with more than 50 frequently occurring drugs and pharmaceutical compounds. Autosampler stability of extracts exceeded a timeframe of 40 h at room temperature for all analytes. The developed method clearly outperformed the previously available GC/MS procedure regarding sensitivity and efficiency.

Application to routine casework samples proved the robustness and efficiency of the method. Since its implementation in June 2023 approximately one thousand DUID cases were analyzed. The majority of samples was tested positive for THC and its main metabolites with THC plasma concentrations ranging from 1.0 to 200 µg/l. A small number of samples was tested positive for CBD (n=33) and HHC (n=6) with concentrations ranging from 1.0 to 27 and from 2.1 to 37 µg/l, respectively.

Conclusion: We present a validated, sensitive and specific analytical method for simultaneous determination of THC, 11-OH-THC, THC-COOH, HHC and CBD in human blood and plasma samples. The method is easy to handle, does not require high-end analytical equipment and allows for reliable differentiation between traditional cannabis consumption and consumption of HHC and/or CBD products. Since its implementation and accreditation according to ISO 17025, the method has proven to be robust and fit for purpose in long-term routine analysis not

only in DUID cases but also within post-mortem samples. Furthermore, the design of the method allows for an easy and uncomplicated extension to further cannabinoids if required, thus being prepared for new challenges within a quickly changing cannabis market including semi-synthetic cannabinoids.

Doping control analysis of perfluorocarbons in equine plasma by headspace gas chromatography-tandem mass spectrometry

AM P–13

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Background & Aims: Perfluorocarbons (PFCs) are blood substitutes due to their high oxygen dissolving ability. As such, PFCs are potential doping agents especially for endurance sports. PFCs, classified as oxygen carriers, are prohibited in horseracing according to Article 6 of The International Agreement on Breeding, Racing and Wagering (IABRW) published by International Federation of Horseracing Authorities (IFHA). PFCs are also banned substances considered to have no legitimate use in horses by the Fédération Equestre Internationale (FEI). In human sports, perfluorochemicals are listed in the Prohibited List (under M1, Manipulation of blood and blood components) by the World Anti-Doping Agency (WADA). In order to control the misuse of PFCs in horseracing and equestrian events, the aim of this project was to develop and validate a sensitive method for detecting seven PFC targets in equine plasma samples. These targets are active components in PFC-based blood substitute products.

Methods: The seven PFC targets to be detected in this study were perfluorodecalin (PFD), perfluoromethyldecalin (PFMD), perfluoro-tert-butylcyclohexane (PFTBCH), perfluorooctyl bromide (PFOB), perfluorodecyl bromide (PFDB), 1,8-dichloroperfluorooctane (DCPFO) and perfluorotripropylamine (PFTPA). Bromoperfluorobenzene (BrPFB) was used as the internal standard. Headspace gas chromatography-tandem mass spectrometry (GC/MS/MS) in the negative chemical ionization (NCI) mode with methane as the reagent gas was employed for their detection. Equine plasma samples were first added with ethanol, potassium hydroxide and the internal standard. The mixture was then subjected to headspace GC/MS/MS analysis, and the PFC targets were detected in Selective Reaction Monitoring (SRM) mode.

Results & Discussion: As in-source fragmentation of the PFC targets was observed during NCI GC/MS/MS analysis, the molecular ions might not be observed for selected PFC targets. Therefore, it is important to select a characteristic and prominent precursor ion from the full scan spectrum of each individual PFC standard. With the optimised SRM transition for each target, limits of detections (LODs) ranging from 0.01 to 3.125 ng/mL were achieved. The method has then been fully validated. The inter-day precisions (% RSD) for the target/IS peak area ratios (PARs) were in the range of 7 % to 12 %, while that for relative retention times (RRTs) were in the range of 0.1 to 0.5 % for all PFC targets. The intra-day precisions (% RSD) for the PARs were in the range of 8 % to 16 %, while that for RRTs were in the range 0.1 % to 0.5 % for all PFC targets. The false hit rate and false negative rate were determined to be 0 %. These validation parameters were deemed acceptable for the method to be used as a qualitative screening method on a regular basis. The method has also been applied to the confirmation of selected PFC targets, as the relative abundances of multiple product ions observed in the spiked plasma sample and the reference standard were within the limits as stipulated in the AORC Guidelines for the Minimum Criteria for Identification by Chromatography and Mass Spectrometry published by the Association of Official Racing Chemists (AORC). The method's effectiveness was evaluated by comparing the current detection of PFC targets with the published pharmacokinetic data for other mammals. Assuming similar pharmacokinetics for horses and other mammals such as rat and human, the present method with LoDs at the low ppb (ng/mL) levels should be more than adequate for monitoring the potential misuse of PFCs in horses.

Conclusion: A simple and sensitive headspace GC/MS/MS method has been developed for the detection of seven PFC targets in equine plasma. The SRM transition for each PFC target was optimised to achieve high sensitivity with LODs as low as 0.01 ng/mL. The method possessed good precision to be used as a qualitative screening method for PFCs on a regular basis. Based on published pharmacokinetic data in other animals, the method should be applicable for the doping control analysis of PFCs in horses. The method could easily be extended to accommodate additional PFCs by monitoring additional SRM transitions. This study may also provide insights in the doping control measures for Prohibited Substances with highly volatile properties in horseracing and equestrian sports.

Automated three-step solid-phase extraction of toxicologically relevant psychotropic compounds and metabolites from plasma followed by LC-MS/MS analysis

withdrawn

AM P–14

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Background & Aims: In Brazil, drugs and illicit drugs are responsible for most of the intoxications reported in cases of suicide attempts (SA). According to the latest data published by the Poison Control Center of the Brazilian State of Rio Grande do Sul, in 2022 these groups of substances were present in 6,726 cases of SA. Illicit drugs and antidepressants were the most frequently implicated substances in suicide attempts, followed by benzodiazepines, analgesics/antipyretics, neuroleptics, and anticonvulsants. Laboratory assays for drugs and metabolites play a crucial role in the management of patients with potentially serious poisoning. The identification of the causative agent can facilitate targeted treatment, thereby enhancing recovery rates. Furthermore, the epidemiological understanding of the most frequently detected compounds in drug poisonings can be used to guide public health policies. Considering the information presented, this study aimed to develop and validate a multi-analyte assay for the measurement of drugs and relevant metabolites in emergency toxicology cases in plasma. This method involves rapid automated extraction phase-solid (SPE) using HLB® sorbent, followed by analysis with liquid chromatography coupled with mass spectrometry (LC-MS/MS).

Methods: Target analytes included amitriptyline, amphetamine, benzoylecgonine, carbamazepine, cocaethylene, cocaine, codeine, diazepam, fluoxetine MDA, MDEA, MDMA, methamphetamine, morphine, nortriptyline, paroxetine, sertraline and venlafaxine. Working methanolic solutions of the analytes had concentrations of 200, 300, 400, 1000, 2000, 2500, 5000, 10000, 15000, and 20000 ng/mL, except for carbamazepine which had concentrations of 10000, 15000, 20000, 50000, 100000, 125000, 250000, 500000, 750000, and 1000000 ng/mL. The working internal standard (IS) solution contained amphetamine-d5, benzoylecgonine-d3, cocaine-d3, diazpam-d5, MDMA-d5, and morphine-d3 at 500 ng/mL, fluoxetine-d6 at 5 µg/mL, and trimipramine-d3 at 150 ng/mL. Calibration and quality control samples were obtained by diluting the working methanolic solutions with blank plasma at a 1:20 (v/v) ratio. 100 µL plasma samples were diluted with 875 µL phosphoric acid 4% in ultrapure water, added 25 µL of the IS, and extracted using Oasis HLB® cartridges in an ASPEC® system. To speed up the sample processing time, the SPE method comprises only three steps: application, washing, and elution with a duration of 24 minutes. The extracts were analyzed using Acquity UPLC I-Class with a column ACQUITY UPLC HSS C18 in a 15-minute chromatographic run. The mobile phase was ammonium formate 5 mM pH 3 (A) and acetonitrile with 0.1% formic acid (B), eluted in gradient mode. In addition to the target compounds, 182 other compounds were qualitatively monitored using multiple reaction monitoring (MRM) transitions and retention times.

Results & Discussion: Method validation followed guidelines from the Food and Drug Administration, intra-assay precision 1.6-10.1% and inter-assay precision 1.5-5.8%, with an accuracy of 94.4%-106.6%. Extraction yields 11.3%-99.9%, with matrix effects -15.9%-13.3%. Freeze-thaw stability -6.1%-11.5%, and 12-hour autosampler stability -10.4%-9.8%. Linearity was acceptable with r-values higher than 0.99. The method was applied to samples from 50 SA patients, with 28 samples showing quantifiable substances. Among these, benzoylecgonine (60.7%), diazepam (50%), and cocaine (17.8%) were the most frequently quantified compounds. These results demonstrate the utility of the developed method for simultaneous quantification and identification of multiple analytes, showcasing suitable characteristics for emergency toxicology applications. The automated SPE procedure was based on a generic and simplified protocol for the HLB® sorbent, reducing solvent volume, and thereby minimizing environmental impact. The dilution of plasma samples with 4% phosphoric acid in ultrapure water increased the free fraction of the analytes, reducing bonding to plasma proteins, and reducing sample viscosity. Besides that, the aspiration of a larger volume by the ASPEC® probe reduces the impact of a small unaspirated volume in the sample tubes in the total aspirated volume. Diluted samples were applied into the cartridge, without conditioning or equilibration steps. The washing step was carried out with 5% methanol in ultrapure water, removing salts and proteins. After washing, the drying was performed, using air from the syringe and nitrogen. In the elution stage, a mixture of acetonitrile and methanol (90:10 v/v) was used because, with their organic properties, it is possible to extract the substance from the cartridge, and in this condition, the phospholipids responsible for the matrix effect are poorly eluted.

Conclusion: In conclusion, this study successfully developed and validated a method for the determination of 18 psychotropic drugs and their metabolites in plasma samples from suicide attempt patients in Brazil. The method, utilizing an automated, fast, and simplified three-step solid phase extraction with HLB® sorbent followed by LC-MS/MS, demonstrated acceptable analytical performance. Among the target analytes, benzoylecgonine, diazepam, and cocaine were the most frequently quantified. Additionally, qualitative monitoring using MRM allowed for the detection of numerous other compounds. These findings underscore the method's efficacy for emergency toxicology applications, offering simultaneous quantification and identification of multiple analytes in plasma samples from suicide attempt cases.

AM P-15

Development of a liquid chromatography-tandem mass spectrometry method for the determination of parabens and bisphenol A concentrations in human hair using activated charcoal and dansyl chloride

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Background & Aims: Parabens (PBs) and bisphenols are endocrine disruptors (EDs) families used in daily life. Reproductive disorders, breast cancer, obesity, hypertension, and asthma are known to be linked to these compounds. Blood and urine are the main matrices used in human biomonitoring, tools for assessing population exposure. However, they are not qualified to evaluate long-term exposure to ED with a short elimination half-life. The hair has been proposed as a possible alternative because it is less sensitive to short-term variations in exposure. This study is the first description of an analysis of the hair with the simultaneous use of charcoal to guarantee a free target of analytes and dansyl chloride to increase sensitivity. The aim was to improve the analytical method for rapid and direct determination in hair samples developed for monitoring long-term exposure to (MeP), ethylparaben (EtP), propylparaben (PrP), butylparaben (BuP) used as preservatives and to a plastic monomer bisphenol A (BPA), analyzing by ultra-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Methods: 50mg of hair was incubated with 1 mL of HAc (acetic acid) 1.5% 38°C for 12h. The hydrolysis supernatant was treated with 60mg of charcoal for 1h under agitation. The samples were centrifuged the supernatant was collected. Centrifuges two more times until the charcoal was removed. 800µL of the supernatant will be enriched with 40µL of spiking solutions and 100µL of internal standard. Two liquid-liquid extraction cycles were performed by 1 mL of ethyl acetate. After each cycle the samples were centrifuged and the supernatants were collected and evaporated at 60°C. 50µL of 100mM sodium bicarbonate buffer and 50µL of dansyl chloride in acetone (1 mg/mL) were added, vortexed, incubated (60°C; 15min) and evaporated. The dry residues were reconstituted in 75µL of methanol and 75µL 10mM aqueous solution of ammonium formate. The column Acquity UPLC BEH C18 was at a temperature of 40°C, the sample at 10°C, with an injection of 10µL. Two mobile phases were used, (A) ultra-purified water with 0.1% formic acid and (B) with 0.1% formic acid in metanol (350µL/min). The total run time was 5.5min. The linearity of the calibration models was evaluated at eight levels, in sextuplicate. Blank hair treated with charcoal was added to the combined calibrator solutions of the analytes to obtain [illegible] concentrations 25; 50; 100; 500; 1000, 2500; 5000; and 10000ng/g for MeP; 2.5; 5; 10; 50; 100, 2[illegible]00; 1000ng/g for BuP and EtP; 5; 10; 20; 100; 200, 500; 1000; 2000ng/g for PrP and 2; 4; 8; 40; 80, 200; 400; 800ng/g for BPA.

Results & Discussion: The procedure was effective in removing the analytes added to the blank in all concentrations. Peak area ratios of the analytes added before washing were lower than 20% of the peak area ratios found on the calibrator of the lowest concentration. The method presented satisfactory linearity above 0.99. Intra-day precisions ranged from 1.60-9.09% and inter-day precisions 1.68-8.75% and accuracy 100.71-108.58%. The stability of the extracts was demonstrated under autosampler for 12 h, with maximum differences of analysis series 10.79; 6.18; 3.88; -6.23, and 1.58% for BPA, BuP, PrP, EtP, and MeP, respectively. Extraction yields were higher than 48.38%. As the hair matrix did not allow the performance dilution integrity evaluation as usually performed in liquid specimens, we tested a smaller amount of hair, 10 mg, of highly concentrated QC and evaluated the accuracy of these QC samples using the usual calibration curves. The accuracy of these QC specimens was acceptable, with 103.61% for MeP, 10.51% for EtP, 107.26% for PrP, 102.13% for BuP, and 10.43% for BPA. For the first time, a method allowing the simultaneous determination with the use of charcoal and derivatization of BPA and PBs. In Brazil, there is no assessment of PBs and BPA in hair, unlike in other countries. After hydrolysis was applied of charcoal was applied to give the best results, producing low intercepts and linear calibration curves for all analytes. The method determines very small amounts of selected compounds in hair samples. Four washing solvents were evaluated, and sodium dodecyl sulftate 0.1% was selected as the most effective avoiding organic solventes that may extract our analytes of interest. The derivatization with used dansyl chloride to enhance electrospray ionization contributed toward the development of a specific method and highly sensitive for rapid and exact determination and direct quantification.

Conclusion: A reliable method has been developed, optimized, and validated to determine BPA and PBs in human hair. The advantage of the present study is the possibility of removing the analytes from the hair matrix and obtaining a free blank. The method involves incubation with HAc 1.5%, use of charcoal in curve and controls, derivatization, extraction with acetyl acetate, and analytical determination by LC-MS/MS. The developed method is sensitive, and accurate and is suitable for the analysis of long-term exposure.

Assessment of false positive rates of immunoassay kits for ecstasy compared to chromatographic method

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Background & Aims: In Turkey, enzyme multiplied immunoassay technique (EMIT) is widely used for urine drug screening analysis. Although positive interferences are expected in immunochemical analysis, in Manisa Mental Health and Diseases Hospital, a significant number of samples tested positive for ecstasy using the EMIT-based kit

AM P-16

(SIEMENS) were found to be false positive when re-evaluated by high resolution liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS-ORBITRAP).

Ecstasy (MDMA) and its metabolite (MDA) have structural similarities with numerous substances such as amphetamine-type stimulants, ranitidine, ephedrine, pseudoephedrine, cathinones, etc. Because ecstasy and/or its metabolite can cross-react with antibodies in the amphetamine screening test kit, urine specimens with true ecstasy positivity are also positive for amphetamines. Therefore, the possibility of "false ecstasy positivity" is strongly considered in specimens in which amphetamine positivity does not accompany by ecstasy positivity.

This study aimed to evaluate the analytical performance of the three immunoassay methods by re-analyzing samples predicted as possible "false positives" at EMIT with biochip array technology (BAT) (Randox) and DRI (Thermo Fisher) compared to LC-MS/MS confirmation.

Methods: The urine sample results of donors aged 18-65 years, admitted to the Toxicology Laboratory of Manisa Mental Health and Diseases Hospital for forensic or clinical drug analysis between December 2023- February 2024 (n=2618) were retrospectively reviewed. These samples were analyzed using EMIT-based Siemens brand drug screening kits on the Siemens ADVIA-1800 autoanalyzer. Among the samples positive for ecstasy (n=349, 13.3%), only samples negative for amphetamines were selected (n=100, 3.8%).

One aliquot of these samples was reanalyzed with Thermo Fisher DRI kit on Siemens ADVIA-1800 and Randox BAT kit on Randox Multistat. Indeed, confirmatory analyses of these samples were performed on a Sciex QTRAP 5500 Plus LC-MS/MS device.

A cut-off value of 500 mg/dL was used for immunochemical methods and LOQ for LC-MS/MS (2.89 ng/mL for ecstasy, MDMA; 2.82 for ecstasy metabolite, MDA). Based on the LC-MS/MS results, the results of the three immunoassay methods were classified as true positive (TP), true negative (TN), false positive (FP) and false negative (FN).

Results & Discussion: Fifty six percent of samples included into the study (n=100) were under judicial supervision and probation, 36% were from the Addiction Detoxification Center, 9% were from the psychiatry clinic.

The confirmatory analysis with LC-MS/MS revealed that 96% of samples were false positives and only four samples were true positives (three of which were among the six samples detected >1000 ng/mL by EMIT). DRI found only 8% positive, of which three were true positives (75% of true positives) and five were false positives. BAT detected one of four true positives (25% of true positives), while no false positives were detected in negative samples. Two LC-MS/MS techniques ORBITRAP and SCIEX QTRAP 5500 plus provided consistent results in 4 true positive samples. One true positive sample was positive by EMIT, but negative by the other two immunochemical methods.

In summary, among the ecstasy-positive samples (n=100), 96% (n=96) were false positives with EMIT. Among these, 5.2% (n=5) were false positives with DRI, while no false positives were identified with BAT. Among the four true positive samples, 75% (n=3) tested positive with DRI, and 25% (n=1) tested positive with BAT.

Based on these findings, the false positive rate of the EMIT-based Siemens kit seems to be relatively high. BAT, which doesn't yield false negatives, appears to be highly specific (100%). DRI had 94.8% specifity. However, due to the limited number of true positive samples evaluated, no conclusions can be drawn regarding sensitivity.

Conclusion: The EMIT-based ecstasy kit from Siemens has a significant false positive rate, which increases the need for confirmation and imposes a financial burden. Since our study is based on possible false positive samples with EMIT, it includes a limited number of cases in terms of true positivity. Our investigation will continue by expanding the sample size to include more true positives.

AM P-17

Stability of Δ9-tetrahydrocannabinol acetate in e-liquid during storage period or during vaping

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Background & Aims: In Japan, Δ9-tetrahydrocannabinol acetate (Δ9-THC-OAc), an acetyl derivative of Δ9-tetrahydrocannabinol (Δ9-THC), was designated as a designated drug under the "Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices" on March 10, 2023, and came into effect on March 20 of the same year. Before the regulation, e-cigarette liquids containing these substances were sold via the Internet and other means in Japan.

Cannabis plant extracts have been prevalent as e-cigarette liquids in the illicit market since around 2016. Δ9-THC is an important psychoactive ingredient in identifying these products as cannabis under the Cannabis Control Act. If all or part of the Δ9-THC detected in a liquid product was produced from Δ9-THC-OAc, there would be a possibility of misjudgment and a hindrance to the prosecution of a drug case. To investigate this possibility, we stored or heated Δ9-THC-OAc under various conditions and checked whether Δ9-THC was produced from Δ9-THC-OAc.

Methods: We tried the following experiments using a retail liquid product of Δ9-THC-OAc containing Δ9-THC as an impurity.

(A) A retail liquid product of Δ9-THC-OAc was stored at 80°C or 30°C for 28 days. The Δ9-THC-OAc liquids was subjected to GC/MS to detect representative diluents, glycerol, propylene glycol (PG) and polyethylene glycol (PEG).

(B) A retail liquid product of Δ9-THC-OAc was stored at 80°C for 28 days in the presence of glycerol, PG, or polyethylene glycol 400 (PEG400).

(C) A cartridge containing the Δ9-THC-OAc liquid was installed in an e-cigarette device, which was heated and vaporized using a syringe connected to the cartridge through a silicone tube.

(D) The Δ9-THC-OAc liquid was put into a test tube and heated at 200 °C or 400 °C for 5 min.

The relative concentrations of cannabinoids in the test solution before and after each operation were measured by gas chromatography with flame ionization detection.

Results & Discussion: None of representative diluents were detected in the retail liquid product of Δ9-THC-OAc. Δ9-THC decreased or disappeared in all cases(A~D) of Δ9-THC-OAc liquid. Cannabinol acetate increased in all cases, and unknown products were also detected by gas chromatography/mass spectrometry (GC/MS). The oxidation reaction rather than transesterification is considered to proceed whether or not Δ9-THC-OAc coexists with glycerin, PG or PEG400 which are alcohol.

Conclusion: The present result showed that Δ9-THC-OAc does not decompose into Δ9-THC during each process.

Development and validation of an analytical method for the detection of 8 cannabinoids by GC-MS and evaluation of cannabidiol (CBD) tablets

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Background & Aims: Cannabidiol (CBD) products are increasing in demand, sold as health supplements, vapes and cosmetics in health stores and over the internet. They are sold with the claim that they provide relief from pain and other health benefits however, many of these products have no, or limited scientific data to support this. It has been reported in scientific literature that both CBD, Δ9- tetrahydrocannabinol (Δ9-THC) and other phytocannabinoids have been identified in these products however no meaningful assessment has been published of how these products vary in dose. Since CBD products were, and continue to be, sold as supplements, they do not need to meet the same standards as pharmaceutical products: there is currently no worldwide legislation standardizing production, dosage, or medical claims with respect to CBD products which has the potential to cause problems to the criminal justice system particularly with respect to roadside drug testing.

The aims of this work were to develop and validate a gas chromatography-mass spectrometry (GC-MS) method for the detection of CBD, Δ9-THC, Δ8- tetrahydrocannabinol (Δ8-THC), cannabigerol (CBG), cannabinol (CBN), cannabichromene (CBC), tetrahydrocannabivarin (THCV) and cannabidivarin (CBDV), using deuterated- Δ9- tetrahydrocannabinol (Δ9-THC-D3). Capsugels® containing powder, with a label stating dose of CBD were purchased to establish if labelling on the containers was correct and if any other phytocannabinoids were present that could have pharmacological implications.

Methods: A GC-MS method was validated according to the ASNI/ASB 036 Standard Practices for Method Validation on Forensic Toxicology (1st edition, published 2019). A Scion GC with Bruker MS instrument was employed, and ran in single quadrupole mode. An Agilent J&W, DB-5ms GC Column (25 m, 0.25 mm ID, 0.25 µm film thickness) was used with the initial column temperature was 100°C, held for 1 min, increased at 25°C/min to 300°C then held for 2 minutes. The instrument was run in positive ion mode, with selective ion monitoring and single quadrupole detection. An 8 neutral cannabinoid mixture, 500 µg/mL in methanol and Δ9-THC-D3, 100 µg/mL in methanol as internal standard (both Cerilliant, TX) were used in the method development and validation studies, as well as for preparation of stock and calibration working solutions.

A solid phase extraction method was developed to extract the phytocannabinoids from the tablets. Each powder was prepared with an internal standard (Δ9-THC-D3) and diluted to approximately 200 ng/mL of CBD in 1 mL of methanol with the labelling on the tablet container used to approximate this concentration. Bond Elut Certify II, 200 mg, 3 mL cartridges (Agilent, CA) were chosen after comparison with other cartridges. The cartridges were conditioned with 3 mL methanol and 3 mL water, equilibrated with 1 mL 100 mM ammonium acetate (pH 3) and loaded with 1 mL of sample. Cartridges were washed with 2 mL of water and 2 mL of 95:5 100 mM hydrochloric acid: acetonitrile (%v: %v) then dried down for 20 minutes. Eluting the phytocannabinoids was performed with 2 mL hexane followed by 3 mL 50:50 hexane: ethyl acetate (%v: %v). The resulting extracts were evaporated to dryness at 60°C, under nitrogen. Reconstitution and derivatization of the samples for analysis was accomplished by adding 100 µL of ethyl acetate and 10 µL BSTFA + 1% TMS, samples were capped and heated at 60°C for 20 minutes. These extracts were then run on the GC-MS and data analyzed.

Results & Discussion: 4 different products were tested with all bottles containing a powdered material inside a Capsugel®. On initial testing for qualitative analysis, it was noted that all tablets contained CBD and some of the other phytocannabinoids included in the validated method. 3 tablets from each bottle were analysed with average values used to calculate the amount of CBD and other phytocannabinoids in each bottle. The labelling on the bottles states that 30 mg of CBD should be present with no mention of other compounds. Initial findings indicate from tablets analysed so far, that CBD amount varies from 9-31 mg, THCV varies from 9-27 mg and CBDV varies from 7-24 mg. Other cannabinoids were identified but not quantified however, the products are being further analysed with SPE to increase the sample size and to include more products.

Conclusion: The initial findings from the tablet analyses are that there is variation in each bottle as well as different bottles from different manufacturers containing variation in the amounts of CBD as well as other phytocannabinoids. This work continues and the data will be used to further investigate the potential toxicological implications of administering CBD preparations containing other phytocannabinoids.

AM P–19

Bioanalysis of amphetamines in alternative matrices using SPE-UHPLC-ESI-MS/MS technique and its application to real samples

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Background & Aims: Abuse of amphetamines has become a serious threat to the law-and-order situation across the globe as a large number of violent crimes are committed allegedly under the influence of these drugs. Interest on alternative matrices analysis for clinical and forensic purpose has recently grown. The aim of this study was to develop and validate a fast and sensitive SPE-UHPLC-ESI-MS/MS method for quantification of amphetamines in oral fluid, hair and nail matrices. The validated method was applied to specimens of fifty patients to quantify amphetamines in these alternative specimens.

Methods: Oral fluid was used as such whereas hair and nail specimens were digested in 0.1N HCl prior to solid-phase extraction, followed by liquid chromatography-tandem mass spectrometry, to quantify amphetamines in these matrices. The chromatographic separation was achieved using an Agilent Poroshell120EC-18 (2.1mm x 50mm x 2.7µm) analytical column at 40oC. Agilent Infinity Ultra high-performance liquid chromatography (1260) with tandem mass spectrometer (6470) was employed for quantification. Mobile phases used for gradient elution mode were LC-MS grade water in 0.1% formic acid (mobile phase A) and LC-MS grade methanol in 0.1% formic acid (mobile phase B). The mobile phase flow rate was set to 0.5mL/min with gradient varying the percentage of methanol linearity: 0.0min, 15%; 0.2min, 15%; 3.0min, 90% and 4.0min, 15%. The injection volume was 5µL with 5.0min acquisition time and 2.0min post-run time.

Results & Discussion: The quantification method demonstrated good linear ranges of 5–2000ng/mg (ng/mL for oral fluid, blood and urine) with ≥0.995% coefficient of linearity in oral fluid, hair and nail matrices. The detection and quantification limits were 2ng/mg and 5ng/mg, respectively, in keratinized and non-keratinized matrices. Intra-day and inter-day precision were 2.4-5.9% and 3.3-7.6%. Mean extraction recovery and ionization suppression ranged 91.5-99.7% and -7.2 to -12.9% in the studied matrices. The applicability and trueness of the method were examined by analyzing quality control and real samples from fifty abusers of amphetamines. The results are as follows:

Table 1 Concentration ranges of amphetamines in patient specimens							
History (Months)	No. of patients	Drug/ Metabolite	Blood (ng/mL)	Urine (ng/mL)	Oral Fluid (ng/mL)	Scalp Hair (ng/mg)	Finger Nail (ng/mg)
≥1 to <3	6	Amphetamine	35-98	57-230	19-39	7-18	5-11
	6	Methamphetamine	193-1580	121-560	26-121	9-21	7-19
	4	MDMA	249-1674	153-446	78-313	6-15	10-11
	4	MDA	65-147	68-336	35-78	Not detected	Not detected
≥3 to <6	13	Amphetamine	66-87	76-107	35-58	13-29	12-33
	13	Methamphetamine	89-1351	97-1106	46-310	11-19	9-16
	7	MDMA	350-1168	165-1737	41-98	8-33	10-25
	7	MDA	45-99	73-150	15-69	5-6	Not detected
≥6 to <9	9	Amphetamine	87-149	59-348	49-161	25-62	33-45
	9	Methamphetamine	231-992	93-1279	105-219	17-38	13-28
	11	MDMA	171-1378	54-1063	90-365	41-69	27-55
	11	MDA	41-53	57-211	38-91	7-11	5-6

Conclusion: Alternative specimens are of utmost significance in clinical and medico-legal cases. Analysis of amphetamines in alternative matrices can provide information complimentary to conventional matrices. Furthermore, this study will guide clinical and forensic toxicologists in interpretation of findings in the case scenario where alternative samples are the only option for analysis.

AM P-20

Comprehensive and sensitive LC-MS/MS method for profiling psychoactive substances in drug-facilitated sexual assault cases

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Background & Aims: Drug-facilitated sexual assault (DFSA) constitutes a significant form of drug-facilitated crime, involving the perpetration of sexual violence against individuals rendered incapacitated or unconscious by psychoactive substances. These substances can profoundly affect consciousness, memory, and judgment. Typically, Central Nervous System depressants, akin to acute ethanol intoxication, induce sensations of sedation, muscle relaxation, confusion, dizziness, anterograde amnesia, and loss of consciousness. Despite the prevalence of DFSA cases, Brazil lacks a standardized protocol for detecting substances implicated in these crimes in biological samples. Furthermore, analyzing DFSA cases proves challenging due to the necessity for low detection limits to extend the narrow detection window. Thus, this study aimed to develop and validate an LC-MS/MS quantitative method capable of analyzing 57 psychoactive substances (including antidepressants, benzodiazepines, antihistamines, opioids, stimulants, and other drugs of abuse) and certain biotransformation products, with detection limits compatible with DFSA investigations. The method was applied to urine samples collected from DFSA victims routinely treated at two Brazilian hospitals.

Methods: The selection of substances for analysis was based on the recommendations outlined in the ANSI/ASB Standard 121 guidelines (2021). With the exception of ethanol, medications, drugs of abuse, and select biotransformation products were analyzed in urine samples (400 µL). These samples underwent enzymatic hydrolysis using beta-glucuronidase from Helix pomatia, followed by extraction with MTBE (tube one). For benzoylecgonine extraction, an aliquot (50 µL) of urine samples underwent protein precipitation with acetonitrile (tube two). Following

agitation and centrifugation, the supernatants were combined into a new tube (tube three), and the contents were evaporated. The samples were then resuspended in 100 µL of mobile phase, and 2 µL were injected into the LC-MS/MS system (LCMS8045, Shimadzu, Kyoto, Japan) equipped with electrospray ionization. Chromatographic separation was performed using a Raptor™ biphenyl column (100 x 2.1 mm, 2.7 µm), with a mobile phase consisting of ultrapure water (A) and methanol (B), both containing 0.1% formic acid (v/v) and 2 mmol/L of ammonium formate, eluted in gradient mode. Method validation was conducted following ANSI/ASB Standard 036 recommendations. Ethanol quantification involved analyzing urine samples (100 µL) using HS-GC-FID after the addition of internal standards (500 µL). The analytical method for ethanol quantification had previously been validated according to the parameters outlined in ANSI/ASB Standard 036 recommendations.

Results & Discussion: The developed method met acceptance criteria, with limits of detection (LD) and quantification (LLOQ) as low as 0.5 ng/mL and linearity from 0.5 to 750 ng/mL. Bias and imprecision values were below 14.3% and 14.8%, respectively. The maximum observed matrix effect was -57.4%, and recoveries were above 5.1%. No carryover or interferences were observed. Autosampler stability studies indicated that all analytes remained stable for 24 hours at 10°C. Analysis of forty-two authentic urine samples using both methods revealed the presence of at least one substance in 26 samples (61.9%). Ethanol was the most predominant substance, detected in 11 samples (42.3%) at concentrations ranging from 0.1 to 1.0 g/dL, followed by THC-COOH in seven samples (26.9%) ranging from 5.7 to above the upper limit of quantification (ULOQ) ng/mL, and cocaine in five samples (19.2%) ranging from 1.5 to above ULOQ ng/mL. Additionally, AEME, benzoylecgonine, cocaethylene, meprobamate, nordiazepam, oxazepam, temazepam, zolpidem carboxylic acid, fluoxetine, amitriptyline, diphenhydramine, nortriptyline, and clonazepam were detected. In nine samples, more than two substances were identified, such as ethanol and benzodiazepines, potentially exacerbating their effects when used in combination

Conclusion: A sensitive method based on simple liquid-liquid extraction, protein precipitation, and LC-MS/MS analysis was developed and validated for the quantification of 57 psychoactive substances in urine. The method exhibited low limits of detection and quantification, adequate linearity, bias, and imprecision. Application of the developed and validated method to authentic urine samples revealed the presence of studied substances at low concentrations, underscoring the necessity for sensitive monitoring methods for drugs potentially facilitating sexual crimes in urine. Additionally, a previously developed and validated HS-GC-FID method successfully analyzed the same authentic samples, with ethanol being the most frequently detected substance.

AM P-21

How important is the method? Influence of the analytical technique on quantification of gamma-hydroxybutyrate in post-mortem femoral blood

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Background & Aims: When interpreting quantitative post-mortem GHB results challenges arise: There are several influencing factors to consider when evaluating GHB concentrations. A possible increase due to post-mortem generation and the endogenous nature of GHB has to be taken into account. One important factor is the origin of blood specimen (heart or peripheral) used for analysis, and storage time and storage conditions should also not be disregarded. To date, a possible influence of the analytical method used for the determination of GHB has not been investigated systematically. The aim of this study was to examine the comparability of post-mortem GHB concentrations in femoral blood measured with three different validated analytical methods.

Methods: Post-mortem femoral blood samples were taken from cases without exogenous GHB involvement and stored at -80°C until further analysis. The GHB measurements were conducted in triplicate by: a) gas chromatography-mass spectrometry (GC-MS) with derivatization (BSTFA) after precipitation, b) gas chromatography-mass spectrometry (HS-SPDE-GC-MS/MS) with prior conversion of GHB to GBL and c) liquid chromatography-tandem mass spectrometry (LC-ESI-MS/MS) with SPE after precipitation. The concentrations were statistically compared by a two-way repeated measures ANOVA.

Results & Discussion: In total, samples from 68 individuals were examined. GHB concentrations in the samples analyzed after derivatization (method a) were <0.50-18.43 mg/L (mean was 3.8 mg/L, median was 3.1 mg/L; mean standard deviation 0.22). The concentrations after the conversion of GHB to GBL (method b) ranged from <0.13 to 21.6 mg/L (mean was 4.0 mg/L, median was 3.2 mg/L; mean standard deviation 0.29). The use of the LC-MS method resulted in concentrations of <0.50-18.50 mg/L (mean was 4.2 mg/L, median was 3.0 mg/L; mean standard devia-

tion 0.17). There was no significant difference between the three analysis techniques (p = 0.108), and no significant difference between the mean values of the GHB concentration measurements regarding the three methods (p = 0.236). In addition, there was no statistical interaction between the three different methods and the measurements (p=0.360).

The results of the triplicate measurements showed a very strong correlation between both GC-MS methods (R2= 0.94). The correlation of the LC-MS with the GC-MS method a) and the method b) showed also a strong correlation (R2= 0.80 and R2= 0.78 respectively). Although, correlation at lower concentrations was better than at higher concentrations (>5 mg/L).

Conclusion: There was no statistically significant difference between the three methods used. The mean values of the measured GHB concentrations showed also no significant difference. No interaction between the three methods and the GHB results could be found.

The results show that there is no significant influence of the analytical method that needs to be taken into account in the interpretation.

Analytical validation of antidepressants determination in wastewater using SPE and GC-MS/MS

AM P-22

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Background & Aims: In recent years, the COVID-19 pandemic has been associated with a significant increase in cases of psychiatric disorders. This phenomenon has been reflected in the rising consumption of antidepressants, representing a potential threat to public health and ecosystems. Simultaneously, there has been an increase in negligence among the population, exacerbating the presence of these medications in wastewaters. Such substances are detected in both metabolite and parent drug forms, with the latter being of greater concern as it indicates improper disposal and inefficiencies in wastewater treatment plants. This study aimed to develop an effective method for detecting serotonin reuptake inhibitors and serotonin-norepinephrine reuptake inhibitors classes of antidepressant drugs (fluoxetine (FLX), citalopram (CIT), sertraline (SRT), paroxetine (PXT), and venlafaxine (VLX)) using solid-phase extraction (SPE) and gas chromatography coupled to tandem mass spectrometry (GC-MS/MS).

Methods: Water samples (100 mL) were spiked with the internal standard (protriptyline at 25 ng/mL) and extracted using OASIS Prime MCX cartridges. Samples were conditioned (2 mL of methanol and 2 mL of ultrapure water), washed (2 mL HCl 0.1 M, in water and 2 mL HCl 0.1 M, in methanol), dried for 15 minutes and eluted (2 mL of 5% ammonium in methanol). After derivatization (N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) and 5% trimethylchlorosilane (TMCS)), the compounds were analyzed by GC-MS/MS in multiple reaction monitoring (MRM) mode.

Results & Discussion: Following international validation guidelines, the method was linear from 0.049 (0.19) to 25 ng/mL, with determination coefficients of at least 0.99, and lower limits of quantification (LLOQ) between 0.049 and 0.19 ng/mL. Intra-day, inter-day, and intermediate precisions, along with accuracies, demonstrated coefficients of variation typically below 15% (20% for LLOQ) and accuracy ranging between 80-85%. Additionally, the procedure yielded recoveries between 9 and 75%, and was applied to 32 real samples from wastewater treatment plants in mainland Portugal, where FLX, VLX, CIT, SRT, and PXT were detected in concentration ranges of 0.08-3.40 ng/mL, 7.46 ng/mL, 0.07–3.25 ng/mL, 0.07–3.68 ng/mL, and 0.33–0.97 ng/mL, respectively.

Conclusion: Notably, this method, employing SPE with Oasis PRIME MCX cartridges and GC-MS/MS. Additionally, this is the first study of its kind conducted in Portugal that determines all these compounds distributed throughout Portugal. It serves as a viable alternative for monitoring these drugs in wastewater samples, emphasizing its environmental significance and helping assessing consumption patterns.

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AM P-23

Composition comparisons of original and counterfeit perfumes

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Background & Aims: Counterfeit perfume of luxury brands have been increased owing to high interest on fragrance and diversity of marketing channels. Composition of counterfeit perfume is changeable defending on maker. Methanol(MeOH) is one of the chemical that is focused on counterfeit perfume analysis, since it is known to be used instead of ethanol(EtOH) in order to reduce the production cost despite of its high toxicity.

Methods: In this study, we have analyzed the compositions of counterfeit perfumes, and those were compared with the original perfumes. For that, we prepared three sets of original and counterfeit perfumes from three different brands(Byredo, Jo Malon, and Masion Margiela). Original perfumes were purchased from certified brand store, and counterfeit perfumes were obtained as evidences from investigation agency in Korea. For analysis, we used Bis(Trimethylsilyl)trifluoroacetamide(BSTFA) derivetization based Solid Phase Microextraction(SPME) Gas Chromatography Mass Spectrometry(GCMS) method.

Results & Discussion: We confirmed different concentration of EtOH on counterfeit perfume compared to the original perfume, but no MeOH was found. Obtained limit of detection(LOD) of MeOH was 0.2 %(v/v) by using BSTFA derivatization based SPME-GCMS method. Other chemicals related to fragrance were confirmed. For example, we have found flavoring chemicals such as limonene, rose oxide, pinene, ocimene, etc. from both types of perfume; however some chemicals were only confirmed from counterfeit perfume (e.g., phthalate, cashmeran, alpha-cetone, etc.) or from original perfume (3-carene, p- and o-cymene, cyclamal, etc.).

Conclusion: BSTFA derivatization based SPME-GCMS method could be proper analysis method compared to direct injection method, since EtOH and MeOH can be overlapped on GCMS chromatogram due to similar molecular weight and retention time. Despite counterfeit perfumes on Byredo, Jo Malon, and Masion Margiela showed no MeOH, but other counterfeit perfume evidence from different brand(e.g. Polo Lauren) showed MeOH.

AM P-24

Fast and efficient sample preparation and separation method for determination of diazepam and its major metabolite from human plasma samples

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Background & Aims: The anxiolytic benzodiazepine diazepam (DZP) was first introduced and licensed in the United States in 1963. It is categorized as a benzodiazepine with both long-acting and fast-acting properties. It is frequently recommended to treat a range of diseases including acute recurring seizures, severe muscle spasms, stiffness related to neurological illnesses, anxiety disorders, and alcohol withdrawal. DZP is highly efficient in relieving symptoms associated with acute alcohol withdrawal, such as restlessness, shaking, drunk hallucinations, and severe delirium tremens. The therapeutic drug monitoring (TDM) of DZP is crucial for observing patients' adherence to treatment. Furthermore, the detection of diazepam and its major metabolite (NDZP) holds significant importance in forensic toxicology due to its potential for misuse. There are several reasons why NDZP was chosen for this study. These; It is a major metabolite, has a longer half-life compared to other metabolites, and TDM studies are mostly carried out based on the ratio of DZP and NDZP. In a chronic dosing study, the steady-state metabolite and parent drug plasma concentrations ratio was found 1.26 (Greenblatt et al., 1981). Similarly, Hiemke (2016) stated that the NDZP/DZP ratio between 0.9 and 1.9 is the normal level. However, Hiemke et al. (2018) reported that the detection of DZP plus NDZP concentration above 3000 ng.mL-1 is the laboratory alert level. Our aim with this method we have developed is to produce a method that gives fast, reliable, and accurate results within the specified limits. The developed method can be utilized for both forensic toxicology and TDM purposes.

Methods: The analysis of DZP and nordiazepam NDZP was conducted using an Agilent HPLC system with a UV detector. A Kinetex F5 column was used for separation. The mobile phase consisted of acetonitrile and phosphate buffer in a 30:70 ratio, set in isocratic mode with a flow rate of 1.0 mL/min. The column thermostat was set to 40°C, the injection volume was adjusted to 5 µL, and the detection wavelength was set at 238 nm. Solutions were filtered and degassed before analysis. A protein precipitation method was used to remove proteins from plasma samples. The

validation experiments ensured selectivity, linearity, recovery, accuracy, precision, LOD, and LOQ. Specificity was evaluated with plasma samples from six individuals. Matrix matched QC samples and calibration samples were prepared at four concentrations including LLOQ and six concentrations respectively. Intra- and inter-day repeatability studies were conducted using QC samples and at the end of the study, accuracy and precision parameters were evaluated. Quantitation was performed accounting for relative peak areas.

Results & Discussion: The linearity of the calibration curves drawn at six different points within the desired plasma drug concentration range is demonstrated by R2 values of 0.9989 and 0.9993, respectively. The LOD and LLOQ values were calculated as 0.066 and 0.2 µg.mL-1 for DZP, and 0.052 and 0.2 µg.mL-1 for NDZP, respectively. Upon examination of the results, it is observed that the accuracy values range from 98.62% to 113.25% for NDZP and from 93.20% to 114.24% for DZP. These values meet the ±15% deviation criterion specified in the ICH and FDA bioanalytical method validation guidelines. The RSD% values obtained in the precision study were calculated as 0.42% to 4.06% for NDZP and 0.97% to 5.39% for DZP. As a result of our analysis of real samples (n=84), NDZP was detected in all samples, while DZP remained below the detection limit in fifteen samples.

Conclusion: As a result of our study, a rapid, accurate, and precise sample preparation and analysis method has been developed. The applicability of the method was proven by applying the developed and validated method to real samples.

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Determination of twenty commonly found compounds in DUI and autopsy cases in whole blood using automated 96-well phospholipid removal plate and UHPLC-MS/MS

AM P-25

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Background & Aims: The Department of Forensic Sciences at Oslo University Hospital annually receives approximately 8800 whole blood samples from driving under the influence (DUI) cases and 2700 autopsy cases. In about 90% of the DUI cases, on average three impairment compounds is found in each sample, and determination of the compounds was done on several methods. A multi-component method covering several drug classes will reduce both analysis time, sample consumption and costs. Using a 96-well phospholipid plate gives the possibility to automate the sample preparation and increase sample throughput. Therefore, this study aimed to develop and validate an automated high-throughput method on 96-well plates on a Tecan robot followed by UHPLC-MS/MS for determination of 20 compounds in whole blood from five different drug classes (opioids, cannabis, stimulants, benzodiazepines and anti-histamines); delta-9-THC (THC), hexahydrocannabinol (HHC), amphetamine, methamphetamine, MDMA, cocaine, benzoylecognine (BE), clonazepam, diazepam, nordiazepam, oxazepam, alprazolam, nitrazepam, morphine, codeine, 6-monoacetyl morphine (6-MAM), oxycodone, tapentadol, ethylmorphine, and doxylamine. The development of the sample preparation possessed some challenging compounds, including THC, cocaine, BE and the amphetamines. The method development results will be presented.

Methods: The DUI whole blood samples were collected in 5 mL BD Vacutainer® Blood Collection Glass Tubes (BD Vacutainer Systems, Franklin Lakes, NJ, USA), which contained 4 mg/mL sodium fluoride and 28 IU/mL sodium heparin. The whole blood from the forensic autopsy cases were collected in 25 mL Sterilin tubes (Sterilin, Caerphilly, U.K.) with 200 mg potassium fluoride. To an aliquot of 100 µL whole blood, 50 µL internal standard and 100 µL EtOH:0.2 M ammonium carbonate pH 7 (30:70 v/v), were added before precipitation with 400 µL ice-cold ACN. The supernatant was filtered through a 96-well phospholipid removal plate, and 1 µL of the filtered sample was injected on an Aquity UPLC I-Class system with a Xevo TQS tandem-quadrupole mass spectrometer (Waters). Gradient elution was performed on a C18 column (50x2.1 mm, 1.7 µm) with MeOH and 5 mM pH 10.2 ammonium formate. The sample preparation time for 96 samples on a Tecan robot was 55 min. and the UHPLC run time was 4.5 min. With the exception of HHC and tapentadol, 13C or deuterated labelled internal standards were used for all the compounds. Quantification was carried out with calibrators without whole blood matrix.

Results & Discussion: The calibration curves, where the low range represents the cut-off, covered the concentration ranges found in DUI samples: 0.0030-0.30 µmol/L for THC and HHC, 0.20-20 µmol/L for amphetamine, methamphetamine and MDMA, 0.05-3.0 µmol/L cocaine, 0.2-2.0 µmol/L BE, 0.0040-1.6 µmol/L clonazepam, 0.20-12.5 µmol/L for diazepam and nordiazepam, 0.60-15 µmol/L oxazepam, 0.010-2.4 µmol/L alprazolam, 0.050-2.5 µmol/L nitrazepam, 0.030-5.0 µmol/L for morphine and codeine, 0.03-0.30 µmol/L 6-MAM, 0.050-5.0 µmol/L oxycodone, 0.10-5.0 µmol/L for tapentadol and ethylmorphine, and 0.020-2.0 for doxylamine. As validation is in

progress, preliminary validation results including four assays with low and high quality control samples (n=9) are presented. Satisfactory between assay precision with RSD in the range 1.2 to 12.1% and accuracy 10.9 to -13.6 % were achieved for all the compounds with the exception of THC (-18.9 %), tapentadol (-18.4 %) and doxylamine (-29.7 %) which showed a bias in the lower calibration range. Method comparison with the existing methods included 168 samples (n=143 DUI samples and n=25 postmortem samples). The presented method showed small deviations when compared to the existing methods and were within ± 15%.

Conclusion: An automated high-throughput method including twenty commonly found drugs in forensic samples was developed. The method is intended to be used in routine forensic analysis of DUI and postmortem whole blood samples.

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A multivariate approach for the optimization of a DLLME procedure for the analysis of GHB in urine samples

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Background & Aims: Gamma-hydroxybutyric acid (GHB) is a commonly employed substance in drug facilitated crimes (DFC), mainly connected with sexual assaults. This use is due to the potent sedative and amnesic effects produced by the drug. After the plasmatic peak, GHB concentrations tend to decrease rapidly due to the short half-life, with urinary levels returning to the endogenous level in 6 to 12h. Consequently, there is the need for reliable analytical methodologies for the proper determination of GHB levels. However, nowadays methodologies should focus not only on their analytical features, but also in the sustainability of the process. This can be achieved by using microextraction techniques such as dispersive liquid-liquid microextraction (DLLME). DLLME is a simple technique based on the interaction between the aqueous sample, an extraction solvent and a disperser solvent which needs to be miscible in both previous phases. The addition of a disperser solvente permits a higher contact surface between the extraction solvent and the sample, increasing the efficiency of extraction. Therefore, the aim of this study was to optimize a DLLME procedure for the analysis of GHB in urine samples, followed by determination using LC-MS/MS.

Methods: The DLLME procedure consisted of the addition of hydrochloric acid solution to the urine sample followed by the rapid addition of a mixture of the extraction solvent (ethyl acetate) and a disperser solvent (acetonitrile). This solution was agitated in vortex and centrifuged, and the organic layer was recovered and evaporated to dryness. The extract was reconstituted in 30 µL of acetonitrile and injected into a LC-MS/MS system. A Nexera UFLC system coupled to a LCMS-8045 triple quadrupole mass spectrometer was used for the analysis. Separation was performed using a Shim-pack Velox SP-C18 (2.7 µm, 2.1 x 50 mm) column eluted in gradient mode with (A) ultrapure water fortified with 0.1 % of tributylamine and 0.03 % of acetic acid and (B) acetonitrile as mobile phases. Total run time was 7 minutes. Detection of GHB was performed in ESI negative mode using three multiple reaction monitoring (MRM) transitions: 103.3>57.1; 103.3>85.0; 103.3>59.1. Optimization experiments were performed using blank urine samples fortified with GHB in a 20 µg/mL concentration. Firstly, variables that could influence the extraction were screened through a full factorial design (24) in order to define the significant ones. These variables were sample volume, extraction solvent volume, agitation time and HCl concentration. Afterwards, significant variables ($p<0.05$) were optimized through a central composite design, with the factors being evaluated in five different levels to achieve the best conditions. All statistical analyses were performed using the Statistica® 10.0 software (Statsoft, USA) and the models were evaluated by ANOVA test and the coefficient of determination (r2).

Results & Discussion: After the investigation through the full factorial design, significant variables were found to be the sample volume, the volume of the extraction solvent, and the HCl concentration. The r2 presented by this model was 0.81. Agitation time was not significant, so the minor value of 10 s of agitation was chosen for further experiments. The significant variables were optimized through the central composite design in the following levels: sample volume (133, 200, 300, 400, and 467 µL), extraction solvent volume (83.25, 100, 125, 150, and 166.75 µL) and HCl concentration (0.13, 0.3, 0.5, 0.7, and 0.87 mol/L). This model exhibited a r2 value of 0.91. Unfortunately, it was not possible to change the disperser solvent (acetonitrile) for other similar options. When employing methanol or ethanol, there was no phase separation, making it impossible to collect the organic phase. Additionally, the extraction solvent (ethyl acetate) was not optimized, since it has already been successfully employed for the extraction of GHB in beverages (Meng et al., 2020). Therefore, the final DLLME procedure was as follows: An aliquot of 350 µL of urine sample was mixed with 50 µL of HCl solution in a 0.9 mol/L concentration. This was succeeded by adding a mixture of 200 µL of acetonitrile and 150 µL of ethyl acetate. The final solution was agitated in a vortex for 10 s and centrifuged at 10000 rpm for 5 minutes. Afterward, the organic layer was collected and followed the previously described procedure.

Conclusion: In this study, a DLLME procedure was successfully developed and optimized for the determination of GHB in urine samples. This sample preparation method is simple, fast, and consumes a low amount of organic solvents, in line with the Green Analytical Chemistry principles. Further experiments will focus on the validation of the developed methodology, respecting the established GHB cut-off value (10 µg/mL) in urine for DFC testing, and the application in real case samples.

Identification of selected substances with a hormonal effect (peptides) using LC – MS and MS/MS methods

AM P-27

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Background & Aims: In the last two years, there has been an increase in the number of users of preparations with a hormonal effect in the Czech Republic, and a large number of these substances were analyzed at the Institute of Criminalistics, Police of the Czech Repubic, Department of Chemistry and Physics. These are small and medium peptides, which are part of the list of prohibited substances issued by the World Anti-Doping Agency from 1 January 2021. At the same time, the distribution of these substances and their storage in large quantities in the Czech Republic is prohibited. For this reason, a new LC-MS/MS method was developed to identify these substances. These are, for example, peptides GHRP (growth hormone - releasing peptides), Hexarelin, Melanotan, BPC 157, PT 141 (Bremelanotide), Thymosin Beta-4 (TB-500), Thymosin Alpha-1.

Methods: For the identification of peptides in commercial preparations, the separation method of liquid chromatography (LC) on the reverse phase was used. The measurement was carried out under the following conditions: column - XBridge Premier BEH C18, 2.1 × 50 mm, 2.5 µm, mobile phase A: 0.1% formic acid in water, B: 0.1% formic acid in acetonitrile, gradient elution, flow rate 0.2 ml/min, injection volume: 5 µl, column temperature 25 °C. Mass spectrometry was used for detection. The measurement was carried out using a mass spectrometer with low and high resolution, both by the method of direct injection into the mass spectrometer (Direct Injection, DI) or in conjunction with liquid chromatography (LC-MS). The measurement takes place in Full Scan mode using electrospray ionization in positive mode, the spectra are measured in the range of 100-5500 m/z. After measuring the MS spectra, the fragmentation spectra of the analytes were measured at different collision energies depending on the size of the peptide (10, 20, 35, 40, 60 eV).

Results & Discussion: A new LC-MS/MS method was developed for the unambiguous identification of peptides GHRP 1, GHRP 2, GHRP 3, GHRP 4, GHRP 5, GHRP 6, Hexarelin, Melanotan 2, BPC 157, PT 141 (Bremelanotide), Thymosin Beta-4 (TB- 500) and Thymosin Alpha-1. The analysis of a significant amount of preparations using the developed method thus contributed to the successful detection of an international organized group engaged in the illegal production and distribution of substances with a hormonal effect.

Conclusion: A significant number of preparations originating from illegal production were analyzed using this method, some of which contained a different active substance than the declared composition. For example, a preparation with the declared active substance BPC 157 contained Melanotan 2, another preparation that was supposed to contain the substance Thymosin Beta-4 (TB-500) contained only mannitol. The use of these home-made preparations could be harmful to the health of consumers. According to information from investigators, the perpetrators' profits from the sale of hormonal preparations were several times higher than the profits from the drug business.

Isolation and determination of combined drugs with antihypertensive effects in biological material (enalapril maleat, reserpine, dihydralazine, hydrochlorothiazide and triamterene)

AM P-28

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Background & Aims: In recent times, practical healthcare is facing with an urgent problem associated with self-medication and an increase in the number of poisonings as a result of improper use of various medications. The increase in the number of types of drugs used in clinics has parallelly led to the growth of the number of complications arising from the use of new, which are not yet sufficiently studied drugs, and from existing ones that are

used in combinations or in large doses. The utilization of large doses of diuretics, mainly the thiazide series, during a long-term therapy of arterial hypertension (AH), contributed to an increase in the number of sudden coronary deaths [1]. Due to the high prevalence of arterial hypertension, the treatment of this disease remains one of the main problems of modern medicine. On the core of the potentially dangerous combinations of therapeutic agents lies drug interaction, leading to a decrease in the safety of pharmacotherapy.

The purpose of this research was a chemical and toxicological analysis of combination drugs such as Carlon Plus (hydrochlorothiazide and enalapril maleat), Adelfan-Ezidrex (reserpine, dihydralazine and hydrochlorothiazide) and Triampur Compositum (hydrochlorothiazide and triamterene) in biological material such as liver, blood and urine.

Methods: The development of a method for isolating hydrochlorothiazide and triamterene in their joint presence from the liver was carried out using the aqueous acetone method, infusing the biological material with a mixture of acetone and water (5:3). Afterwards, the acetone extracts were combined and the acetone was evaporated. To separate and purify the preparations from matrix substances, followings were performed: the remaining aqueous part, after evaporation of the acetone, was acidified with a 0.1 M solution of hydrochloric acid and hydrochlorothiazide was extracted with ethyl acetate three times in 10 ml portions. The ethyl acetate extracts were separated and evaporated to dryness. The aqueous part was made alkaline with 0.1 N sodium hydroxide solution and triamterene was extracted with chloroform three times (10 ml each). Chloroform extracts were also separated and evaporated to obtain a dry residue. The resulting dry residues were dissolved in a small amount of mobile phase (acetonitrile - 1% acetic acid solution, 30:70) and analyzed by HPLC.

To develop a method for isolating adelphan-esidrex, the aforementioned extraction method was deployed with aqueous acetone, taking into account the pH values during extraction from both acidic (hydrochlorothiazide) and alkaline solutions (dihydralazine at pH 9.0 and reserpine at pH 10). Chloroform and ethyl acetate extracts from acidic and alkaline solutions were separately examined by TLC and UV spectrophotometry. For quantitative determination, eluates were spectrophotometered at the maximum wavelength's characteristic of the drugs: for hydrochlorothiazide at λmax = 272 nm, for reserpine at λmax = 265 nm and dihydralazine at λmax = 274 nm. The corresponding solvent was used as a reference solution. The amount of test substances in the resulting eluates was calculated according to the calibration graph.

Results & Discussion: For purification and preliminary detection of the test substances, the TLC method was deployed via a Silufol 254 plate. Satisfactory separation of the ingredients making up Carlon Plus was observed in a solvent system consisting of isopropanol-ethyl acetate-chloroform-glacial acetic acid (4:5:7:0.2) with optimal Rf values of 0.68 for hydrochlorothiazide and Rf of 0.32 for enalapril maleate. For the separation of hydrochlorothiazide and triamterene, the most optimal system is a mixture of chloroform and ethanol (4:1). The spots were developed under UV light. Moreover, the Rf values of hydrochlorothiazide and triamterene are 0.65 and 0.42, respectively. The best separation of Adelphan-Ezidrex components was observed using the chloroform-ethyl acetate-methanol system (9:2:1). UV irradiation at 254 and 366 nm was used to detect localization zones of hydrochlorothiazide (0.30), dihydralazine (0.47) and reserpine (0.19). For the analysis of Triampur Compositum via HPLC, the same conditions were used that were developed for the analysis of hydrochlorothiazide: Zorbax XDB C-8 column (150 x 4.6 mm) model 1100 Series from Agilent Technologies, UV detector at 272 nm, mobile phase: acetonitrile - 1% acetic acid (30:70), flow rate - 1 ml/min, column temperature at room temperature. In this case, the retention time is 3.59 (triamterene) and 4.81 (hydrochlorothiazide). For chromatography of the Carlon plus preparation, detection was carried out at 215 nm, mobile phase: buffer solution (pH 2.2) - acetonitrile (75:25), flow rate - 2 ml/min, column temperature - 50°C. The retention time of hydrochlorothiazide is 2.29, enalapril maleate is 1.26 minutes.

Conclusion: The method for the isolation and determination of combined antihyoertensive drugs such as Carlon Plus, Triampur Compositum and Adelfan-Ezidrex in biological material has been developed. The proposed method makes it possible to isolate hydrochlorothiazide and triamterene from biological material in their joint presence by an average of 64.0 and 54.16 percentages. On the other hand, in the case of analyzing Adelphan-esidrex, the proposed method allows to isolate dihydralazine, hydrochlorothiazide and reserpine from biological material by 52.0, 60.54 and 26.48 percentages respectively.

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One-step extraction procedure for the analysis of tetrahydrocannabinol Δ9- and Δ8- isomers and metabolites, hexahydrocannabinol, and selected minor phytocannabinoids in human whole blood

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Background & Aims: Cannabis remains one of the most popular recreational drugs worldwide. Δ9-tetrahydrocannabidiol (Δ9-THC) is the main psychoactive agent of cannabis and plays a major role in driving under the influence of drugs (DUID) investigations. Therefore, Δ9-THC and its metabolites are generally subject to legal thresholds regarding driving. In recent years, further phytocannabinoids, e.g. cannabidiol (CBD), and (semi-)synthetic cannabinoids like hexahydrocannabinol (HHC) and Δ8-tetrahydrocannabinol, have emerged on the licit and illicit drug markets. This has led to the need for new methods covering these cannabinoids as well. Analytical methods for the determination of Δ9-THC and other cannabinoids in blood samples often require laborious multi-step cleanup, concentration, and/or derivatization procedures. The presented study aimed to develop and validate a rapid, robust, and straightforward method in micro blood samples for the separation and quantification of Δ9-THC and Δ8-THC, CBD, their respective metabolites, as well as HHC and a subset of further phytocannabinoids in whole blood.

Methods: A one-step sample preparation procedure was developed in a 96-well plate format using only 50 µL of whole blood. The blood was precipitated with a mixture of 150 µL methanol:acetonitrile (1:1 v/v) containing the internal standards. After mixing and centrifugation, the sample plate was placed in the autosampler, and the supernatant was analysed. The method was validated on an LC-40D XR ultrahigh-performance liquid chromatography (UHPLC) system (Shimadzu, Kyoto, Japan) hyphenated to a QTrap 6500+ triple quad mass spectrometer (AB Sciex, Massachusetts, USA). Chromatogrpahy was performed with a Kinetex XB-C18 (50 x 2.1 mm, 1.7 µm) core-shell column (Phenomenex, California, USA) operated at 50 °C within 10 minutes. Mobile phases consisted of water (mobile A) or methanol (mobile B) both supplemented with 0.1% formic acid. The method was validated in accordance with regulatory guidelines. Parameters evaluated included method linearity, working range, recovery and matrix effects, accuracy and precision, stability, and limits of detection and quantification (LODs and LOQs) for a total of 14 cannabinoids (Δ9-THC, Δ9-THC-OH, Δ9-THC-COOH, Δ8-THC, Δ8-THC-COOH, CBD, CBD-6-OH, CBD-7-OH, CBD-COOH, Δ9-THC-acid (THCA), tetrahydrocannabivarin (THCV), cannabigerol (CBG), cannabinol (CBN), and HHC).

Results & Discussion: The application of a one-step sample preparation procedure significantly reduced the workload for sample cleanup. The validation criteria were fulfilled for all analytes, resulting in a fit for purpose analytical method reliably covering Swiss legal thresholds for Δ9-THC and respective metabolites in whole blood. Excellent recoveries (≥88%), highly consistent matrix effects across seven blood donors (%CV: ≤7%) and suitable LODs and LOQs were achieved. For Δ8-THC, Δ9-THC, and Δ9-THC-OH the LOQs were 0.5 ng/mL, whilst for Δ8-THC-COOH and Δ9-THC-COOH the LOQs were 2.5 ng/mL. For all analytes the LODs and LOQs ranged from 0.2 - 0.8 ng/mL and 0.5 - 2.5 ng/mL, respectively. A subset of "minor" phytocannabinoids (CBD, CBG, THCV, THCA, CBN) was included in the method, due to their proposed role in supporting data interpretation in forensic casework. Finally, the method includes recently emerged HHC and Δ8-THC, adapting to current market developments. The presented method allows for the baseline separation of Δ8-THC-COOH and Δ8-THC from the corresponding Δ9-THC isomers. However, it is not possible to distinguish Δ8-THC-OH from Δ9-THC-OH. Therefore, the hydroxy metabolite should be expressed as the sum of both isomers.

Conclusion: The presented method is in line with current efforts to minimize sample volume, organic solvent consumption, workload, and costs, while adapting to recent developments such as the emergence of products containing Δ8-THC and HHC. Further, automatization of the sample cleanup procedure and application of the method to other sample matrices, such as urine and dried blood spots, is feasible without significant effort. During method validation the presented method proved fit for purpose, as Swiss legal thresholds are well covered. This study demonstrates that sample cleanup for comprehensive cannabinoid analysis in blood does not always require extensive procedures. A simple precipitation procedure can achieve the necessary specifications for a quantitative bioanalytical method.

Sensitive determination of cannabis constituents in oral fluid by LC/MS-MS

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Background & Aims: Cannabis sativa L. stands out as the most widely used illicit drug globally, consumed at rates comparable to legal substances like tobacco and alcohol. Despite being included in the list of drugs in Schedule I of the United Nations Single Convention on Narcotic Drugs in 1961, its medicinal properties have gathered public attention. Active secondary metabolites in cannabis have shown efficacy in treating various conditions such as

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chronic pain, cancer pain, depression, and anxiety. This increased interest has prompted legislative changes in many countries, impacting public health and the production, use, and sale of cannabis.

As the cannabis market expands, there is a continuous need for updated analytical methodologies to monitor this growth. These methods analyze a wide range of cannabinoids and metabolites in different samples to assess cannabis consumption. Such analyses are crucial for distinguishing between legal and illegal use and determining active component concentrations.

The importance of studying non-conventional biological matrices like oral fluid (OF) is gaining recognition. OF samples offer advantages such as the non-invasiveness of its collection, ease of collection by non-medical personnel, reduced risk of adulteration, and the likelihood of containing parent drugs, reflecting recent consumption, which is a significant advantage of this matrix.

Methods: Herein, we present an analytical methodology developed in OF samples for the determination of tetrahydrocannabinol (THC), 11-hydroxy-tetrahydrocannabinol (THC-OH), 11-carboxy-tetrahydrocannabinol (THC-COOH), cannabinol (CBN) and cannabidiol (CBD) by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), aiming at documenting cannabis consumption. Briefly, 200-µL OF aliquots undergo protein precipitation with a refrigerated methanol/acetonitrile mixture (80:20, v/v). After centrifugation, the extracts are evaporated to dryness, reconstituted in methanol, and 5-µL aliquots are injected into the UPLC-QTRAP-MS 6500+ (SCIEX®) system with a total runtime of 14 minutes. The analysis operates in multiple reaction monitoring (MRM) mode, with two transitions monitored for each compound and one transition for each internal standard. The method was fully validated following the guidelines of ANSI/ASB 0362 Standard Practices for Method Validation in Forensic Toxicology.

Results & Discussion: Results showed that the method produced linear outcomes for all compounds tested. The working ranges were determined as 0.1–50 ng/mL for THC, 0.5–50 ng/mL for THC-OH, CBN, and CBD, and 0.05–1 ng/mL for THC-COOH. While ion suppression was observed for THC, CBN, and CBD, it did not affect sensitivity due to the low limits of quantification (LOQs) and limits of detection (LODs) obtained, ranging between 0.05 and 0.5 ng/mL. The extraction procedure yielded high recoveries, and the compounds were stable. No interferences were found.

Conclusion: The developed methodology provides a valuable tool to assess recent cannabis consumption using a non-conventional biological matrix whose advantages have been gaining recognition. The method proved to be extremely fast, robust, selective, precise, and accurate for use in routine analysis. Notably, the method achieved a LOD forty times lower and a LOQ twenty times lower than the THC screening cut-off value of 2 ng/mL recommended by the Substance Abuse and Mental Health Services Administration (SAMHSA) for OF samples. The method was successfully applied to authentic samples.

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Development of a chromatographic method for the analysis of flumethrin causing prethyroid toxicity

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Background & Aims: Varroa destructor, a common bee pest, can cause significant bee losses worldwide. Acaricides used to control and combat this bee parasite are extremely limited. Flumethrin, a pyrethroid used against the Varroa destructor mite, is excessively and incorrectly utilized worldwide. As a result of this unintentional use, it causes toxicity-related harm to honey bees. It can accumulate in high amounts in bee products particularly honey due to its lipophilic nature (If the combs are not replaced regularly, they become the source of contamination while extraction of the honey). Flumethrin residue degrades the quality of bee products while also posing a substantial risk to public health due to the deaths caused by pyrethroid toxicity. Therefore, detecting flumethrin residue is critical, but the available detection methods are limited. In this project, a fast, selective, and sensitive method will be developed for flumethrin using HPLC-UV, an alternative to traditional methods with new-generation columns. Optimised method applied to honey and commercial human plasma samples to demonstrate the applicability of the developed HPLC methodology.

Methods: A novel HPLC-UV method was developed utilizing a Kinetex EVO C18 (Phenomenex, USA) analytical column (150 mm × 4.6 mm i.d., 5 µm). The mobile phase consisted of 0.1% acetic acid adjusted to pH 2.7 and acetonitrile (20:80 v/v), with a constant flow rate of 1 mL/min. The column temperature was set to 40°C. Detection was performed at a wavelength of 267 nm, and samples were injected at a volume of 20 µL.

Results & Discussion: The validation of the method was conducted in accordance with the guidelines set by the International Council for Harmonization. The limits of detection (LOD) and quantification (LOQ) were established using signal-to-noise ratios. For both compounds, the LOD and LOQ were determined to be 0.03 µg/mL and 0.05 µg/mL, respectively. As a result of the real sample applications on honey two out of ten samples included flumethrin in LOD level.

Conclusion: The developed extraction and determination method offers a reliable and selective approach for determining flumethrin from commercial plasma samples and honey samples supplied from local beekeepers without any interference. This project is still continue and the honey samples will be received from different regions of Türkiye. Until now, 2 out of 10 samples included flumethrin. Because of that reason, this methodology can help monitoring flumethrin as an acaricide which is widely used around the world.

A new approach to analysing immunosuppressant drugs and creatinine using a dried blood spot (DBS) autosampler with offline LC-MS/MS analysis: Preliminary data comparison with electrochemical luminescence immunoassay (ECLIA) for kidney transplant patients

AM P-32

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Background & Aims: DBS analysis, based on capillary blood sampling by finger prick, is a rapidly emerging technique for the therapeutic drug monitoring (TDM) analysis of clinical blood samples. It offers significant avantages over standard methods based on venous blood sampling. These include: a less invasive patient sampling experience, sample stability and the prospect of "self-sampling" by the patient in the comfort of their own home.

The overall aim of this work was to conduct a clinical study to directly compare immunosuppressant (ISUPP) drug and creatinine concentrations in blood samples from kidney transplant patients using two different sampling and analytical methods. Venous blood samples were analysed using an established immunoassay method, whilst capillary (fingerprick) blood samples were measured with a newly developed dried blood spot (DBS) /liquid chromatography/tandem mass spectrometry (LC-MS/MS) method. The DBS method used a fully automated DBS autosampler operating in "offline" mode. The analytes of interest were Cyclosporin A, Tacrolimus, Everolimus, Sirolimus and creatinine. Individual aims for the DBS method were: to achieve the required clinical concentration reference ranges for all analytes, to fully validate the method and to assess its merits as an alternative to the established method.

Methods: DBS samples were obtained in a clinical setting by finger prick. Cards were allowed to dry overnight at room temperature before being delivered to the analytical laboratory. DBS cards were extracted using a fully automated DBS autosampler (CAMAG, Muttenz, CH). The DBS autosampler measured hematocrit values for each DBS non-destructively, in real-time prior to extracting cards. 200µL Methanol/water (80:20 % v/v) extracts were collected using a PAL liquid autosampler (CTC Analytics AG, Zwingen, CH). After evaporation and re-constitution, the extracts were analysed by isotope dilution-LC-MS/MS on a Sciex 6500 QTRAP system (Sciex, Framingham, MA).

Venous blood samples were analysed for immunosuppressant drugs by ECLIA and for creatinine with the Jaffe colorimetric test, using a COBAS 8000 (Roche, Mannheim, DE)

Data comparison was by Deming regression.

Results & Discussion: Tacrolimus, Everolimus and Sirolimus were quantifiable in the range 1.5 (the lower limit of quantification, LLOQ), to 100ng/mL; the Cyclosporin A range was from 15 (LLOQ) to 1000ng/mL. The method was fully validated. Using a limited sample number from preliminary data, a linear regression (R2 =0.9293) showed excellent agreement for the Tacrolimus concentrations obtained from DBS (LC-MS/MS) and venous blood (ECLIA) methods. By means of an additional LC-MS/MS analysis of the DBS extracts, quantitative data for creatinine was obtained (this dataset will also be correlated with data from the established method).

Conclusion: The DBS method has been developed to deliver maximum flexibility; the DBS sample extracts are collected in a microtiter plate and in principle can be analysed on any available validated LC-MS/MS platform. The offline nature of the DBS extraction method allows the sensitivity enhancement demanded by the clinical reference range to be obtained. In addition to Cyclosporin A, Tacrolimus, Everolimus and Sirolimus, the method, with minor modifications, was adapted to provide quantitative creatinine data.

Tacrolimus concentrations obtained with the DBS method showed excellent correlation with those obtained using an immunoassay method.

The method should lend itself well to patient home-/self-sampling.

AM P–33

Quantification of drugs in oral fluid using LC-MS/MS: A study of detection windows

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Background & Aims: At our laboratory, we have a method for quantification of 28 drugs in oral fluid. During method development, the measuring ranges for the analytes were set based on expected concentrations in oral fluid available in the literature at that time, but limited by the sensitivity and linear range of the analytical instruments.

We recently performed a study of detection windows in oral fluid of single therapeutic doses of 16 medicinal drugs with addictive properties. Doses were ranging from 100 µl fentanyl to 100 mg tramadol. A number of samples collected eight hours after intake of highly potent or rapidly eliminated drugs showed concentrations below the lowest calibrator. Therefore, we made an attempt to lower the limits of quantification for six of the analytes in the method: morphine, methylphenidate, tapentadol, ketobemidone, fentanyl and zolpidem.

Methods: Quantisal Oral Fluid Collection Device was used for sample collection. This gives a dilution of the oral fluid of 1+3 in the collection device. The sample preparation was a semi-automated liquid-liquid extraction using ethyl acetate and heptane with the use of a Hamilton automated liquid handler. The final reconstituted extract was about two times the concentration in the collected samples. The original method was fully validated. The simplest way to lower the limit of quantification without making drastic changes to the method was to use a more sensitive detector.

The original method was validated on an Acquity UHPLC from Waters with a BEH C18 column (2.1 x 5 mm, 1.7 µm, Waters) and a Xevo TQ-S triple quadrupole MS detector (Waters). 5mM ammonium formate pH 10.1 (A) and methanol (B) were used as mobile phases in a linear gradient of 5% B at 0.00 min, 5% B at 0.15 min, 30% B at 0.30 min, 50% B at 2.70 min, 90% B at 3.80 min, 98% B at 4.80 min and 5 % B at 4.81 min with a flow of 0.5mL/min. In the attempt to lower the limit of quantification, a Xevo TQ-XS detector was used, with the same MSMS parameters. Xevo TQ-XS is more sensitive and has a wider linear range compared to Xevo TQ-S. Several different concentration levels below the original lowest calibrator were tested, and signal to noise ratio (S/N), linearity and precision was assessed.

Results & Discussion: The lowest concentration levels which gave S/N > 10, linear curves and precision within ± 15% from nominal value was chosen as the new lowest calibrator. The limit of quantification was lowered for morphine (from 3 to 0.3 ng/mL), methylphenidate (from 0.3 to 0.06 ng/mL), tapentadol (from 10 to 1.0 ng/mL), ketobemidone (from 1.0 to 0.2 ng/mL), fentanyl (from 0.1 to 0.02 ng/mL) and zolpidem (from 0.3 to 0.03 ng/mL).

The relevant study samples were reanalysed together with a calibration curve including a calibrator at the new LOQ level. This gave quantifiable results for all analytes among all study participants, which led to valuable information and increased the detection windows in oral fluid for multiple

Conclusion: The study of detection windows proved that our method's current limits of quantification were set too high to reliably detect intake of therapeutic, single doses for several addictive medicinal drugs.

By lowering the limit of quantification for morphine, methylphenidate, tapentadol, ketobemidone, fentanyl and zolpidem, we were able to quantify all these drugs in all subjects ≥ 8 hours after intake and, in most cases, ≥ 24 hours.

AM P–34

Misuse of pregabalin, prospective and analytical study

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Background & Aims: The misuse of psychoactive medicinal substances has emerged as a new form of drug addiction worldwide. The objective of this study is to develop and validate an analytical technique for assessing pregabalin using LC UV.

Methods: This is a prospective analytical study conducted in 2023, focusing on a series of urinary samples collected from individuals seeking treatment at detoxification centers in Constantine and Setif, cities located in the eastern region of Algeria. A total of 133 patient samples were screened.

Urine samples are analyzed upon arrival using VIVA-E for the detection of cannabis, barbiturates, amphetamines, and cocaine. Additionally, an immuno-chromatographic rapid test is employed to detect tramadol and pregabalin.

We followed the method described by Rajinder Singh Gujral et al. (2009) for the analytical development of urine pregabalin screening by HPLC-UV. All urine samples were analyzed using a LC UV system (Shimadzu). The separation was conducted on an ultra C18 5 µm column (150 mm × 4.6 mm) with a mobile phase consisting of methanol, acetonitrile, and 0.02 M dihydrogen potassium orthophosphate (K2HPO4) (pH 7.00) in a ratio of 3:1:16 (v/v/v). The flow rate was maintained at 1.0 ml/min

Results & Discussion: Out of the 66 subjects who admitted to taking pregabalin, the rapid urine test showed 38 positive results, while the LC UV analysis yielded 43 positive results.

We compared the immunochemical technique with the chromatographic method by calculating sensitivity and specificity using the following formulas:

Sensitivity = True Positive / (True Positive + False Negative) = 38 / (38 + 5) = 88.4%Specificity = True Negative / (True Negative + False Positive) = 88 / (88 + 0) = 100%

Conclusion: It is recommended to conduct initial screenings at psychiatric centers to assist in the diagnosis and appropriate treatment of drug addiction. Understanding the limitations of urine screening through EMIT and rapid tests is essential for their optimal utilization. Chromatographic methods serve as the reference standards for identifying and quantifying pregabalin.

The rapid detection of adulterated, substituted and/or synthetic urine in drugs of abuse screening programs

AM P-35

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Background & Aims: Urine samples are sometimes adulterated or substituted by persons when they are submitted for drug screening by enzyme immunoassay (EIA). Some means of urine sample adulteration can be detected by the first-generation urine sample validity tests (SVTs - pH, creatinine, specific gravity and oxidants), but enterprising groups have invested significant amounts of time and money to sell products that defeat the first-generation urine SVTs. We developed and evaluated a rapid panel SVT (Validity Diagnostics - VDx GEN2-SVT™) to determine if the rapid panel SVT can detect more adulterated samples and to detect more substituted and/or synthetic urine samples when compared to the first-generation urine SVTs.

Methods: On an automated clinical analyzer, the VDx rapid panel SVT [True Urine LD, True Urine SD and Oxidant History] was used to evaluate urine samples for adulteration, dilution, use of oxidants and use of synthetic urine or substituted urine. As required by various drug screening programs, we also used several first-generation SVT reagents to test urines for pH, creatinine, oxidants and specific gravity. Approximately 500 urine samples from pain management patients were tested over a three-month period. Using the VDx rapid panel SVT, urine samples which gave results of less than 10 units on any one or more of the three rapid panel SVT tests were flagged for further evaluation by an SVT algorithm we developed to determine if the urine sample was adulterated, diluted, synthetic or substituted. Using the first-generation SVT reagents, urine samples flagged as being outside the reference range were also flagged for further evaluation.

Results & Discussion: The validation of the VDx rapid panel SVT involved testing blind urine samples provided by Validity Diagnostics in the validation kit. The VDx rapid panel SVT correctly identified all subverted samples which included 18 adulterated urines, 11 commonly used synthetic urines and 10 substituted urines (urine of another, pooled and frozen urines). The adulterated urines tested included the addition or use of: Acid; Alkali; Sodium Acetate; Stealth Oxidant; Chromium; Bleach; Nitrite; Gluteraldehyde; p-Chloromercuriben; Pyridinium Chloride; Protease; Buffer pH 3.3 or pH 8-9. The commercially available synthetic urines tested included: Perfect Urine; Dr Green Super Hero; Quick Fix Plus; Ultimate Gold; Ultra Pure; Dr Greens X Agent; Golden Flask; Gator Wizz; Monkey Flask; Synthetix5; and UPASS.

During the three-month study of approximately 500 urine samples from pain management patients, the VDx rapid panel SVT identified 47 urine samples that had indicators of subversion (adulterated, substituted or synthetic urines). Using the first-generation SVT methods (pH, Creatinine, Specific Gravity, Oxidants), only 15 urine samples were identified as having indicators of subversion.

The retrospective analysis of more 10,000 urine specimen results from several clinical laboratories using the VDx rapid panel SVT showed that ~ 10 - 15% of urine specimens had indicators of subversion (adulterated, substituted or synthetic urines) and were considered "invalid" for subsequent urine drugs of abuse (DOA) screens. This contrasts with ~ 0 - 5% urine specimens showing subversion by the first-generation methods.

Conclusion: Some adulterated, substituted or synthetic urines may not be detected by the first-generation urine sample validity tests (pH, creatinine, oxidants and specific gravity) without additional testing. The VDx rapid panel SVT increases the effectiveness of pre-analytical SVTs to include 15+ classes of subversion (adulteration, substitution, synthetic urine, etc.) commonly used by individuals to "pass" a urine drug screen. Urine specimens passing the VDx pre-analytical validity tests can undergo urine DOA screening with more confidence. Urine samples from donors showing indicators of subversion by the VDx rapid panel SVT should be considered for testing by LC/MS-MS to possibly identify the subversion method or to broaden the number of drugs detected.

AM P-36

Simultaneous screening of 129 illicit drugs and metabolites in urine using GC-MS/MS

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Background & Aims: Forensic investigation has played an indispensable role within the framework of the drug-related criminal justice system. Drug testing methods using biological samples have been extensively explored in a variety of studies. However, there has been limited development of simultaneous quantitation methods for a broad range of drugs of abuse (DOAs) in biofluids. Herein, we developed the simultaneous analysis method to detect and quantitate 129 DOAs and metabolites in 100 µL of urine using GC-MS/MS.

Methods: A total of 129 illicit drugs and their metabolites were targeted in this method. We selected recently abused drugs as target compounds based on a review of both domestic and international literature. For sample preparation, three different methods were compared: QuEChERS, LLE-acid (Ethyl acetate with Formic acid), and LLE-base (Ethyl acetate with NH4OH). One hundred microliters of urine were used for each analysis. The validation of the method followed the guidelines in the Standard Practices for Method Validation in Forensic Toxicology (ANSI/ASB Standard 036, 1st Ed. 2019), assessing specificity, sensitivity, linearity, matrix effect, and accuracy and precision for both intra- and inter-day measurements.

Results & Discussion: The QuEChERS method demonstrated the highest efficiency among the three sample preparation methods in all validation parameters. The validation results of the optimized QuEChERS method are as follows: Out of 129 compounds, 99 had low LOQs (0.1 - 1.0 ng/mL), 27 had LOQs between 1.1 and 6.0 ng/mL, and 3 had LOQs between 7.9 and 13.3 ng/mL, with all 129 compounds showing coefficient of determination(R2) greater than 0.984. The intra- and inter-day precision remained below 20% for all targeted compounds at the concentration of 10 ng/mL. A total of 79 compounds demonstrated accuracy ranging from 75% to 125%. 23 and 24 compounds showed accuracy below 75% and over 125%, respectively. Additionally, the LLE-acid method exhibited better recovery in detecting propofol.

Conclusion: In this study, the simultaneous analysis method for 129 illicit drugs using GC-MS/MS followed by QuEChERS was proposed and demonstrated high sensitivity and robustness. The convenience of this method lies in its quick extraction method using QuEChERS without derivatization. In addition, only a small volume of urine is required. The developed method could be employed for drug testing in forensic investigations, enabling rapid screening of a wide range of illicit drugs and their metabolites.

AM P-37

Determination of 59 neuropsychiatric drugs, including antidepressants, antipsychotics, ADHD drugs, anti-alcohol abuse drugs, and antiepileptics, and their metabolites in urine using LC-MS/MS for medication compliance monitoring

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Background & Aims: Increasing social concern about mentally disordered offenders focuses attention on the need to find ways to reduce their recidivism. One of the most important strategies used to cope with that issue is the establishment of the medication compliance monitoring system. It guarantees that mentally disordered offenders under supervision adhere to their prescribed medication, and plays a significant role in preventing recidivism and endorsing social reintegration. In this study, we investigated and validated a method for determination of the 59 neuropsychiatric drugs, including antidepressants, antipsychotics, ADHD drugs, anti-alcohol abuse drugs, and antiepileptics, and their metabolites in urine using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Methods: Urine samples were prepared using the dilute-and-shoot approach. The sample preparation procedure is as follows: first, 50 µL of urine was mixed with 50 µL of the internal standard solution (10 ng/mL) and 1,900 µL of H2O:methanol (9:1, v/v) solution. Then, the mixed solution was centrifuged for 5 min at 50,000 g and the supernatant was injected into the LC-MS/MS system (Waters Xevo TQ-XS with Acquity UPLC I-Class). Target compounds were separated using a Waters Xselect HSS T3 column (2.1 × 150 mm, 2.5 µm particle size), and a gradient elution was achieved using 0.1% formic acid in water and 0.1% formic acid in methanol at a flow rate of 200 µL/min.

Results & Discussion: All compounds were separated and analyzed within 11.5 minutes. The developed method was validated by evaluating the selectivity, limits of detection (LOD), lower limits of quantification (LLOQ), linearity, accuracy, precision, matrix effect, stability, and dilution integrity according to the U.S. FDA Bioanalyticl Method Validation guidance. The LODs varied from 0.12 to 1.34 ng/mL, and the LLOQs were determined to be 1.0 or 5.0 ng/mL. The correlation coefficients (r) were consistently above 0.995. The intra- and inter-day accuracies ranged from -9.0% to 10.5% and -12.0% to 9.3%, respectively, while the precisions ranged from 0.5% to 8.8% and 1.2% to 10.6%, respectively. The stabilities of the bench-top, long-term, and auto-sampler were also verified, showing percentages ranging from −9.2% to 3.5%, -15.0% to 11.6%, and -11.0% to 12.2%, respectively. The results of dilution integrity revealed an accuracy range of -6.4% to 12.2% and a precision range of -6.4% to 9.1%. Selectivity and matrix effect were also met to criteria. Forensic urine samples (n=248) from mentally disordered probationers were analyzed to demonstrate the practicability of the developed LC-MS/MS method, and determine 53 neuropsychiatric drugs and their metabolites.

Conclusion: An LC-MS/MS method was developed and validated for simultaneously determining 59 neuropsychiatric drugs and their metabolites in urine, and successfully applied for medication compliance monitoring of mentally disordered probationers. Therefore, the developed method is expected to be used as an effective and practical means to strengthen the management of mentally disordered probationers and to reduce the recidivism of mentally disordered criminals.

AM P-38

Analysis of amphetamine-type stimulants in urine by headspace solid-phase microextraction: Derivatization problem

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Background & Aims: Amphetamine-type stimulants (ATSs) are the second most commonly used substances worldwide. Therefore, their analysis plays an important role in the struggle with the substance abuse. This requires reliable and sensitive analytical methods in line with the relevant legal regulations for toxicological applications. Recent studies focus on green analytical method development for reducing solvent consumption and working with small sample volumes. Solid-phase microextraction (SPME) method is one of the miniaturized techniques that provides a strong reduction or even absence of toxic organic solvents. In this technique, semi-volatile analytes like ATS could be converted into volatile and thermally stable derivatives with better chromatographic behavior by reacting with a suitable reagent. Commonly used reactions for this purpose are silylation, alkylation, and acylation which can be performed by the addition of the reagent into the sample matrix, or post-extraction in another closed vial, called on-fiber derivatization. In the present study, a green method based on headspace solid phase microextraction (HS-SPME) of ATSs in urine samples followed by detection with gas chromatography-mass spectrometry (GC-MS) was developed. The critical role of the on-fiber derivatization was evaluated.

Methods: A HS-SPME method was developed for amphetamine (AMP), methamphetamine (MA), 3,4-methylenedioxy amphetamine (MDA), and 3,4-methylenedioxy methamphetamine (MDMA) using a polydimethylsiloxane/divinylbenzene (PDMS/DVB, 65-µm, 24 Ga) commercial fiber. The fiber was first placed into the headspace of a urine sample for adsorption of semi-volatile ATSs and then, the fiber was transferred into another vial where the acylation reaction was performed with a reduced amount of derivatization agents in comparison to literature to retain the fiber stability. The efficacy of different derivatizing agents namely pentafluoro propionic anhydride (PFPA), acetic anhydride (AA), and trifluoroacetic anhydride (TFAA) was compared concerning fiber stability. For determining the stability, the method was performed with these agents at the derivatization step and effectiveness of agents was evaluated in terms of durability, sensitivity and peak symmetry. The parameters affecting the method were screened by Plackett-Burman design for sample volume, salt amount, mixing speed, equilibrium time, adsorption time, derivatization time, and temperature. The method was validated according to the guidelines of the Standard Practices for Method Validation in Forensic Toxicology (SWGTOX) including selectivity, linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), matrix effect, and carryover.

Results & Discussion: The performance of three different acylation agents has been searched and the best results were obtained with trifluoroacetic anhydride (TFAA). Fiber deterioration was avoided by using a reduced amount of reagent in another vial for derivatization and it was concluded that this step has also assisted complete desorption of the analytes from the fiber in the GC-MS system. Screening results obtained with the Plackett-Burman design revealed that adsorption time, derivatization time and temperature were the most effective factors. To determine the most effective factors of the method, regression analysis (ANOVA) was performed according to the peak area values obtained at the end of each experiment. The best response for all the analytes was obtained by exposing the PDMS/DVB fiber for 10 min onto 1.0 mL of urine samples containing 0.50 g of Na2CO3 equilibrated at 80oC and post-derivatization with 25 µL of TFAA in another vial for 1 min. The temperature in derivatization step was tested between 60-80oC and higher derivatization temperatures were avoided since its contribution to the reducing fiber life and response over time and worse reproducibility. The method was found to be selective for amphetamine, methamphetamine, MDA and MDMA. The calibration studies performed have revealed that the graphs obtained were linear within the concentration range of LOQs–2000 ng/mL for all the analytes. The coefficient of determination R2 was >0.99. The resulting LODs values were found comparable with previous studies. Analytical recoveries were greater than %75 for all substances at low and high concentration levels. Precision values were within the acceptable range with a maximum deviation of 9.68% (MDMA). The calculated matrix effects were 0.53 (AMP), 0.63 (MET), 0.61 (MDA) and 0.65 (MDMA). No contamination was detected as a result of the carryover study.

Conclusion: A simple, user-friendly and solvent-free HS-SPME method was developed and allowed the simultaneous extraction of ATSs. Minimal use of acylation reagent has reduced the degradation of the fiber and provided well-formed chromatographic peaks for all ATSs examined, probably due to enhanced desorption of analytes strongly adsorbed on the fiber.

AM P–39

Experimental design to optimise the derivatisation of cannabinoids and benzodiazepines

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Background & Aims: Design of experiments (DOE) is a statistical approach to optimize reactions to modify different factors simultaneously in order to determine optimal factor levels.

The aim of this work is to determine the best conditions for the derivatisation of cannabinoids and benzodiazepines for analysis by GC MS.

Methods: 1-Cannabinoids derivatisation: the full two-factor experimental design with three central points was chosen, the two factors used were the volume of derivatisation reagent (BSTFA-1% TMCS) with a minimum of 20µL and a maximum of 80µL and the derivatisation time with a minimum of 10min and a maximum of 20min, the central points with a volume of 50µL and a time of 15min. The temperature was set at 70°C.

2-Benzodiazepines derivatisation: the full two-factor experimental design with three central points was chosen, the first is the volume of derivatisation reagent (MTBSTFA-1% TBDMCS) with a maximum of 80µL and a minimum of 20µL, the second is the oven temperature with a maximum of 90°C and a minimum of 70°C, including three central points with a reagent volume of 50µL and a temperature of 80°C. The reaction time is 30 minutes.

Results & Discussion: For the derivatisation of Cannabinoids, the optimum is obtained for a derivatisation reagent volume V=50µL and a duration of 15min.

For Benzodiazepines, the optimum derivatisation reaction is obtained for a volume of 80µL and a temperature of 70°C.

Conclusion: The experimental design enabled us to define optimal derivatisation conditions with a limited number of experiments.

Only the volume of derivatisation reagent had a statistically significant influence on the results.

AM P–40

Analysis of over 200 drugs and metabolites in blood and urine by LC-MS/MS for DUID, DFC, and postmortem investigations

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Background & Aims: As driving under the influence of drug (DUID), drug-facilitated crime (DFC), and postmortem casework becomes more abundant and increasingly complex, forensic toxicology laboratories are often pushed to develop processes that are more efficient, broader in scope, and more sensitive, all while on a limited budget and keeping turnaround times low. Further, as novel psychoactive substances (NPS) come in and out of prevalence, the scope of testing must expand to accommodate these changes.

The aim was to develop an LC-MS/MS method for the detection of over 200 drugs and metabolites in blood and urine in DUID, DFC, and postmortem investigations, with a secondary aim of creating a subset of drugs to be quantitated in blood. The method required validation, accuracy, and sensitivity sufficient to meet national standards and recommendations for the three types of casework.

Methods: The extraction method utilizes 0.15 mL of blood or urine, and a protein precipitation with size-exclusion filtration, followed by evaporation and reconstitution prior to analysis by LC-MS/MS. Target analytes include 206 drugs and metabolites including amphetamines, analgesics, anesthetics, anticonvulsants, antidepressants, antihistamines, antipsychotics, barbiturates, benzodiazepines, cannabinoids, cardiac drugs, cathinones, cocaine and metabolites, decongestants, dissociatives, hallucinogens, miscellaneous drugs, muscle relaxants, opioids, and Z-drugs. Targets were assessed qualitatively in urine. In blood, the most prevalent analytes in authentic casework (119) were assessed quantitatively, while the others were assessed qualitatively.

Results & Discussion: The performance of the developed method was assessed by a comprehensive validation protocol, ultimately meeting ANSI/ASB Standard 036 method validation requirements. Limits of detection met sensitivity recommendations set by the U.S. National Safety Council's Alcohol, Drugs and Impairment Division's Recommendations for DUID investigations, and were suitable for other forensic casework including postmortem and DFC investigations. Accuracy and measurement of uncertainties were also appropriate, particularly important in DUID casework.

Method validation studies identified analytes that may undergo conversion into or interfere with the identification of other target analytes. As a result, target analytes were divided into two sub-mixes in which spiked standards such as limits of detection, calibrators, and quality controls can be analyzed in the absence of the interfering analyte.

The extraction method was developed on the Hamilton VANTAGE automated liquid handler, increasing efficiency and throughput within the laboratory. However, in the event of instrument maintenance or downtime, the extraction method can alternatively be performed manually by the analyst, improving versatility in everyday laboratory operations.

Conclusion: The large number of targets from a wide range of drug classes allows the laboratory to stay informed on current drug prevalence within the population, and to keep up with the ever-changing NPS landscape in real-time. By increasing analytical scope, a laboratory may run fewer assays and batches, saving on both time and cost. Combined with the use of automated liquid handling, this highly efficient and robust process streamlines DUID, DFC, and postmortem casework within the laboratory in a time-sensitive manner.

The development and validation of a LC-MS/MS method for the analysis of 17 antipsychotic drugs in whole blood, using a phospholipid removal sample clean up step.

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AM P-41

Background & Aims: Antipsychotic drugs (AP) are prescribed for the treatment of schizophrenia and psychosis. APs are broadly classified as first-generation APs (i.e. chlorpromazine and haloperidol), second-generation APs (clozapine, olanzapine, quetiapine) and third-generation APs (i.e. aripiprazole). They differ in their side effects, with first-generation APs being associated with extrapyramidal side effects; second generation APs can lead to metabolic syndrome, requiring monitoring and the third generation, although noted to have less side effects, are still associated with akathisia (a movement disorder)1. APs are considered of interest in forensic casework due to their abuse potential and involvement in overdoses, or the implications with non-compliance of prescribed medication.

The prescribing of more than one AP is a practice performed to achieve the control of adverse symptoms associated with APs2. Therefore, in a forensic setting, it is beneficial to have a method that can quantify multiple APs in one blood sample.

The aim of this study was to develop and validate a LC-MS/MS method for the determination of 17 antipsychotic drugs in whole blood, which cover a wide range of first-, second- and third- generation APs. The aim was to explore the use of protein precipitation and phospholipid removal as simple clean up steps to produce a quick and easy sample preparation method.

Methods: Whole blood samples, 200 µL, containing 17 APs and an AP internal standard mix were subjected to protein precipitation by the addition of 0.8 mL acetonitrile with 0.1% formic acid. Following centrifugation, the supernatant was transferred to 1mL PhreeTM (Phenomenex, UK) phospholipid removal cartridges and passed through under positive pressure. The eluent was collected, dried down and reconstituted in mobile phase for analysis by LC-MS/MS. An ACE Excel 2 C18-PFP column (Hichrom, UK) was used under gradient conditions with mobile phases consisting of 0.5mM ammonium acetate in water (with 0.1% formic acid) and methanol. An Agilent 1290-6420 UHPLC triple quadrupole mass spectrometer was operated in the positive ESI mode under dynamic MRM conditions.

Results & Discussion: An LC-MS/MS method for the determination of 17 APs in whole blood was developed and fully validated.

The 17 APs were split into two sub-groups, based on their therapeutic ranges. A low calibration range for chlorpromazine, flupentixol, fluphenazine, haloperidol, paliperidone, prochlorperazine, reboxetine, risperidone, trifluoperazine, zuclopenthixol was set at 1 to 50 ng/mL (R2 ≥ 0.990). A high calibration range for amisulpride, aripiprazole, clozapine, norclozapine, olanzapine, quetiapine and sulpiride was set from 5 to 250 ng/mL (R2 ≥ 0.990).

Dilution studies were conducted which showed up to a 1 in 50 pre-extraction dilution can be performed to effectively extend the concentration ranges to 2500 ng/mL for the lower calibration range compounds and 12,500 ng/mL for the high concentration range compounds. An approximate concentration is available for common APs such as clozapine and quetiapine, which are pre-screened on a different LC-MS/MS method and an appropriate dilution factor can be selected to ensure results lie on the calibration range.

Intra-and interday precisions were investigated at low (3ng/mL), medium (12ng/mL) and high (40ng/mL) QC concentrations for the low calibration range and at low (15ng/mL), medium (60 ng/mL) and high (200ng/mL) QC concentrations for the high calibration range. Both intra-day (within-run) precision and inter-day (between-run) precision was less than 15% at all QCs levels and ranged from 0.7 to 14.5 % and 2.0 to 13.9 % respectively.

Whole blood samples which are only subjected to protein precipitation can contain significant levels of interferences which are chromatographically difficult to resolve and hinder the ability to reach required detection limits. Phospholipid removal cartridges were used for a fast method of reducing the levels of interferences without requiring further sample preparation following protein precipitation.

Chromatographic separation was initially attempted using a commonly used C18 stationary phase however problems were encountered with poor retention and coelution with remaining matrix interferences. The PFP (pentafluoro-phenyl) modified C18 phase facilitates lipophilic interactions between the C18 chains and the aromatic regions of the AP and additionally dipole-dipole interactions with the halogenated functional groups.

A further benefit to the use of a PFP column was the strong retentive mechanisms between the LC column and the drugs, with elution requiring a relatively high percentage of the organic phase, allowing efficient electrospray desolvation, greatly increasing sensitivity and an elevated mobile phase flow rate allowing a faster run time (all analytes elute within 6.5 minutes).

Conclusion: A simple and fast preparation of whole blood samples has been developed, along with a selective and quantitative LC-MS/MS analysis for a range of antipsychotic drugs. The use of phospholipid removal cartridges has allowed for low limits of detection without any further sample preparation.

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AM P-42

Comprehensive drugs analysis in blood by phospholipid depletion and orbitrap LC-MS

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Background & Aims: The analysis of drugs in blood is a critical component of both forensic and clinical investigations. However, conducting a comprehensive drug analysis presents challenges due to the wide variety of drugs and their properties such as pKa and LogP. Currently, our laboratory employs a method for the extraction of basic drugs (generally Log P > 1) under alkaline conditions using Supported Liquid Extraction (SLE), followed by LC-QTOF-MS analysis. This panel allows for the quantitation of over 90 drugs, including common anesthetics, antidepressants, antihistamines, benzodiazepines, narcotics, stimulants, beta-blockers, phenothiazines, and SSRIs. A

complementary technique for the analysis of acidic and neutral drugs is thus required. This study focuses on investigating sample preparation using phospholipid depletion (PLD) and analysis by Orbitrap LC-MS. PLD is not pH dependent and can extract drugs with Log P < -1, enhancing the detection and quantitation of a broader range of drugs compared to SLE. The Orbitrap LC-MS, a high-resolution mass spectrometer, utilizes a mass spectral library for drug identification. It is highly selective and sensitive, making it an optimal choice for comprehensive drug analysis.

Methods: For sample preparation, 0.5 ml of blood is 'crashed' using cold acetonitrile and the phospholipids are removed using Agilent Captiva EMR-Lipid cartridge, followed by elution using acetonitrile/water (4:1), and reconstitution in 100 µl of methanol. Analysis is performed on a Phenomenex Kinetex Biphenyl column (100 mm x 2.1 mm i.d, 2.6 µm) attached to a Vanquish UHPLC system with an Orbitrap Exploris 120 HRMS with an ESI source. Gradient elution is achieved using aqueous 0.1% formic acid (mobile phase A) and 0.1% formic acid in methanol (mobile phase B). MS detection, with polarity switching, is based on full scan data dependent acquisition with inclusion list. The criteria for the positive identification of a drug are mass error < 5ppm, delta retention time within ±0.25 min, isotope pattern similarity >80% and library match score >70. Method validation of 80 quantitative drugs was done in accordance with ANSI/ASB Standard Practices for Method Validation in Forensic Toxicology. The target drugs include common analgesics, NSAIDs, antibacterial, antidiabetics, antiepileptics, angiotensin II receptor antagonists, diuretics, and PDE-5 inhibitors. Parallel studies with >90 case and proficiency test samples were conducted to verify that results are comparable to existing methods.

Results & Discussion: PLD successfully extracted all 80 drugs in this study with relative recovery >70%, except for 3 drugs (50% to 70%). Seven deuterated internal standards with retention times that span the entire LC run and of various drug classes were used to monitor the extraction and instrumental analysis. Ten matrices were studied for selectivity and no interference was found for the drugs of interest and the internal standards. Matrix effects at low and high concentrations were calculated in terms of %CV of relative ionization suppression/enhancement and were found to be in the range of 5% to 19% for all drugs except amiloride (18% and 21%) and glimepiride (18% and 30%). Additional matrices (nine instead of three) were tested to check that the lower limits of quantitation (LLOQ) of amiloride and glimepiride were not affected. Linearity was investigated over five days and found that weighted regression using 1/x or 1/x2 was necessary. Bias (-19% to 20%) and precision (2% to 16%) results based on triplicates of quality controls spiked at low, medium, and high concentrations were acceptable. The limit of detection (LOD) was determined to be sufficiently low for the purpose of clinical and forensic investigations. Dilution integrity using a ratio of 1:5 had bias of -20% to 10% and within-run CV of 5% to 13%. Processed samples kept in the refrigerator at 4°C were generally stable for at least six days. Proficiency test results were within ±20% of the assigned values. On top of these 80 drugs, Orbitrap mass spectral library contains >700 toxicologically relevant drugs which can be search against for simultaneous qualitative identification. The parallel studies showed consistency with previously detected drugs. In addition, >100 basic drugs detected by SLE with LC-QTOF-MS analysis were also detected in this PLD with Orbitrap LC-MS method.

Conclusion: The 80 drugs encompassing diverse polarity from Log P < -1 (e.g. metformin, gabapentinoids) to Log P ~5 (e.g. sibutramine) and pKa values from ~3 (e.g. salicylic acid) to ~11 (e.g. amantadine), have been successfully validated. With the ability to simultaneously quantify these 80 drugs and search against a mass spectral library of >700 drugs, PLD extraction with Orbitrap LC-MS analysis thus complements our existing SLE-LC-QTOF-MS technique for comprehensive drug analysis.

Estimating the time of last propofol administration based on the pharmacokinetics of propofol and its metabolites

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Background & Aims: In recent years, propofol carries a high risk of acute toxic poisoning with fatal consequences as well as a high potential for abuse. The study of the pharmacokinetics of propofol and its metabolites, especially the phase I and phase II metabolites, are helpful for the identification of misuse or abuse of propofol in clinical and forensic applications. Based on our previous forensic toxicokinetic studies on diazepam and alcohol, suggesting that the metabolic kinetics of phase I/II metabolites can provide a research basis for determining the time of ingestion. Therefore, this study aimed to investigate the pharmacokinetics of propofol and its metabolites (phase I: 4-hydroxy propofol (4-OHP), phase II: propofol glucuronide (PG)) in blood of Bama pigs, and to provide a predictive model for identifying propofol use and estimating the last propofol administration time.

Methods: Five female healthy Bama pigs, each were injected intravenously with a single propofol dose of 2.2 mg/kg. Blood samples (2 mL) were collected at different times (0, 2, 5, 10, 15, 30, 60, 90, 120, 180, 240, 300 and 360

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min) after the propofol injection, and the collections were extracted through liquid-liquid extraction. Propofol was analyzed by GC-MS/MS with electronic impact (EI) mode (HP-5MS UI capillary column (0.25 mm × 30 m × 0.25 μm)) using thymol as an internal standard (IS); 4-OHP was detected by GC-EI-MS/MS (HP-5MS UI capillary column (0.25 mm × 30 m × 0.25 μm)) following N-methyl-N(trimethylsily) trifluoroacetamide (MSTFA) derivatization and PG was analyzed by LC-MS/MS using electrospray ionization (ESI) in negative multiple response monitoring (MRM) mode (Agilent SB-C18 (3.0×50 mm, 2.7 μm) column; the mobile phase consisted of 50% mobile phase A (methanol) and 50% mobile phase B (ultrapure water) and the flow rate was always 0.25 mL/min). Pharmacokinetics model and pharmacokinetics parameters of propofol and its metabolites (4-OHP and PG) were analyzed by WinNonlin software. The predictive models were constructed for estimating the time of last propofol administration through the relationship between the concentration ratios of propofol to metabolites and metabolites to metabolites, and the predictive performance of the best predictive model was evaluated across clinical 80 patients who took propofol for anesthesia in painless gastroscopy.

Results & Discussion: The mean blood concentration-time profiles of propofol and its metabolites in Bama pigs were in accordance with a two-compartment open model and the pharmacokinetics parameters were calculated with the software of WinNonlin according to the compartmental method. The maximum blood concentration (Cmax) of 11.907±3.448 μg/mL of propofol could generate immediately within 1 min after administration, while 4-OHP had a Cmax of 1.794±0.123 μg/mL at about 28 min and PG had a Cmax of 1.039±0.052 μg/mL at about 18 min. The distribution half-life (t1/2α) and elimination half-life (t1/2β) of propofol were 0.721±0.113 min and 155.703±25.307 min; of 4-OHP were 19.424±74.188 min and 235.824±154.684 min; and of PG were 30.287±14.613 min and 1120.268±648.137 min. The best predictive model was obtained from the concentration ratio of propofol to phase I metabolite 4-OHP and the time of propofol administration with a good correlation, which was successfully verified by 80 real clinical patients for estimating time of last propofol taking with low prediction errors (less than 20%) (within 30 minutes of propofol administration).

Conclusion: In this study, the pharmacokinetic model and parameters of propofol and its phase I/II metabolites were established, and the change rule of drug use time was elucidated, which will provide a sensitive and robust method for the identification of propofol abuse. More importantly, the concentration relationship of propofol and its phase I metabolite was successfully utilized to infer the drug use time, exhibiting considerable potential applications in clinical drug monitoring and forensic illegal drug-related identification.

Poster gallery - NAA P-1 to P-18

16:00 - 16:30 Tuesday, 3rd September, 2024

NAA P-01

About challenges in enantioselective analysis of new psycoactive substances on the example of clephedrone

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Background & Aims: 4-chloromethcathinone (4-CMC) is more popular on the illicit drug market under the name of clephedrone. Since that time 4-CMC is one of the most persistent drugs of abuse in Europe and still regularly found in clandestine laboratories, seized materials and biological matrices of abusers, although it has now a status of controlled substance/drug in many countries. Only in few studies the metabolites of 4-CMC have been also mentioned mostly from the viewpoint of avoiding false negative due to rapid metabolism or perhaps instability of 4-CMC in blood and some other biological fluids. To the best of our knowledge until now no method has been published on enantioselective determination of 4-CMC and its phase I metabolites in human biological fluids. No data were available about possible enantioselective metabolism and pharmacokinetics neither in-vitro nor in-vivo. As some studies report, and we have confirmed in the present study, 4-CMC and its positional isomers are unstable, especially in blood and in some other biological matrices. In this study we report our efforts to develop an enantioselective method for detection one of the drugs of abuse clephedrone and its phase I metabolites in human biological fluids. The major goal is not just reporting our results but preferentially to emphasize the problems/challenges that have still to overcome in order to have a reliable analytical method for detection and quantification of NPS and their metabolites in the matrix of interest.

Methods: Analyte separation was achieved on an HPLC 1290 Infinity II (Agilent Technologies, Germany) coupled to a mass spectrometer equipped with an electrospray ionization source (ESI) operated in positive mode. Autosampler and column oven temperatures were 25° C. MS/MS-detection optimization was conducted in part automatically

and in part manually. MS parameter settings were optimized by ramping cone voltage and collision energy. Mass spectrometric parameters for detection of 4-CMC and its phase I metabolites are summarized in Table 1. Scan speed (dwell time) was 0.93 sec. ESI conditions were optimized as follows: capillary voltage 3500 V, source temperature 300°C, cone gas flow rate 10 L/min, desolvation and gas flow rate 12 L/min.

Results & Discussion: The identification of possible 4-CMC phase I metabolites in the OF and urine of one of the volunteers under this study led to a detection of some phase one metabolites of 4-CMC. Specifically metabolite N-1(C9H10ClNO)(N-Demethylation product) and metabolite-N-2(C10H14ClNO)(β ketoreduction product) and metabolite N-3(C10H13ClNO2)(ω Hydroxylation product) were identified. Of these metabolites N-2 and N-1 seem to be the major metabolites while the metabolites N-3 and N-5 (C9H12ONCl) (β ketoreduction and N-Demethylation product) were detected in minor amount or not detected at all in the studied OF sample. Since just one metabolite (N-1) of 4- CMC was commercially available. Thus, this preliminary enantioselective method for analysis of 4- CMC and its metabolites was developed by using real biological samples and the standard was used only for native compound 4-CMC. Instability of synthetic cathinones and specifically also of 4-CMC is quite well known. This phenomenon was observed also in our study, such as absence of 4-CMC in OF within 1.0-1.5 hrs after its administration, its fast disappearance from blood and longer presence in OF and urine. Various kinds of stability experiments were performed. The initial sample concentration in all studied 3 matrices were 100 ng/ml and one set of samples were kept in a freezer at -20 °C and other set was kept at a room temperature (23 °C). The signal of 4-CMC disappeared from the blood sample kept at room temperature just within 33 hrs. This means that blood samples need to be immediately frozen after sampling. Otherwise, they should be used for clinical-toxicological or forensic analyses performed for identification or quantification of 4-CMC with a great care (or not be used at all). 4-CMC was more stable in OF and urine although significant degradation was observed also in these matrices within a few days.

Conclusion: Bioanalysis of new psychotropic substances (NPS) represents significant challenge due to absence of related standards, especially for metabolites. When NPS is chiral then additional challenges are caused by the absence of enantiomerically pure standards, as well as due to significant increase the number of species (enantiomers and stereoisomers) to be separated. If the NPS is a synthetic cathinone then the stability of the parent compound and metabolites in biological matrices and solvents used for sample preparation and separation creates additional problems. All of these issues have to be carefully considered in order to develop a reliable enantioselective bioanalytical method for simultaneous separation of a new NPS and its pharmacologically relevant metabolites in biological matrixes. Some of these problems were highlighted in the present work on the example of 4-CMC and in few cases possible solutions to these challenges are discussed.

UHPLC-MS/MS method for the separation of non-deuterated and partially deuterated enantioisotopologues and isotopomers of amphetamine

NAA P-02

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Background & Aims: In the recent decades, the isotope effect has particularly captured the interest of scientists in the biomedical field. Compounds containing deuterium undergo slower transformations, leading to favourable metabolic and pharmacokinetic properties and lower toxicity of biologically active deuterated compounds. These advantageous properties could be exploited not only in pharmaceuticals, agrochemicals and electronics but also in illicit drug laboratories. Isotopologues and isotopomers have very similar physical and chemical properties, making their complete or partial chromatographic separation difficult. The main objective of this work was therefore to study the isotopic effect of non-deuterated amphetamine and some partially deuterated amphetamines: amphetamine-d5 (deuterium in the side chain), amphetamine-d5 (deuterium in the phenyl moiety), amphetamine-d6, amphetamine-d8 and amphetamine-d11 and to separate these isotopologues and isotopomers, using various achiral columns. Since amphetamine and its deuterated analogues are chiral compounds, simultaneous separation of isotopologues, isotopomers and enantiomers was another objective of the study using polysaccharide-based chiral columns.

Methods: Separation was performed on a HPLC 1290 Infinity II coupled to a mass spectrometer (6470A Triple Quadrupole LC-MS) equipped with a jet stream electrospray ionization source operating in positive mode. Analysis of all compounds were performed on three chiral and two achiral columns and isocratic elution mode was adopted in all experiments. Lux Cellulose-3 (based on cellulose tris(4-methylbenzoate)) and two Lux AMP columns from Phenomenex were used as chiral columns. Lux Cellulose-3 and one of the Lux AMP column had the dimensions 250

x 4.6 mm, while the other Lux AMP was 150 x 4.6 mm. All three achiral columns (Kinetex 2.6 µm Phenyl-Hexyl and Kinetex 2.6 µm Biphenyl) were of 100 × 2.1 mm dimensions. In case of Lux AMP columns, which are unique columns with pH stability in the range 1.0–11.5, methanol with 0.1 % (v/v) ammonium hydroxide was initially used as mobile phase; while later the mobile phase was composed of methanol and 5 mM ammonium bicarbonate (pH=11.0, adjusted with ammonium hydroxide) in water in the ratio 95/5 (v/v). After this, the ratio was decreased to 20/80 (v/v) of methanol and 5 mM ammonium bicarbonate aqueous solution. The same procedure was used with acetonitrile as mobile phase organic component. In the case of the other three columns, the same approach was used, but using the native pH of the 5 mM ammonium bicarbonate solution (pH 7.7). For the Lux AMP columns, 1 mL/min mobile phase flow rate was used. In the case of the achiral columns, since these columns have highest number of theoretical plates on 0.4 ml/min of mobile phase flow rate it was chosen accordingly.

Results & Discussion: The separation of isotopomers was very limited and in some cases almost non-existent in all columns tested and with all mobile phases and ratios. Using methanol combination with 0.1% (v/v) ammonium hydroxide aqueous solution as mobile phase, the isotopic effect between the isotopologues was also very limited in both achiral and chiral columns. Same results were observed in acetonitrile on every tested columns. With the addition of 5% ammonium bicarbonate buffer, a "normal" but still weak isotopic effect was observed between isotopologues, in acetonitrile on every column. Same picture was observed in methanol on every column but not on Lux AMP chiral column. On Lux AMP column "inverse" isotope effect was observed on low ratio of ammonium bicarbonate buffer in the mobile phase, after increasing buffer ratio in the mobile phase degree of isotope effect (resolution between isotopologues) was increased. Based on the chromatographic resolution, the best separation results for isotopomers and isotopologues were obtained using acetonitrile-ammonium bicarbonate type mobile phases, the best separation of amphetamine enantiomers was obtained with the 25 cm long Lux AMP chiral column in combination with methanol and 5 mM ammonium bicarbonate in water, ratio of 50/50 (v/v) with resolution (Rs) 3.01.

Conclusion: Strong isotope effect was observed for partially deuterated AMP derivatives under some conditions enabling their baseline separation on achiral and polysaccharide-based chiral columns in HPLC. The nature (positive or negative) of the isotope effect and its extent strongly depends on the nature of selector/adsorbent, medium and structure of studied compounds. On chiral columns some correlations were observed between the strength of isotope effect and selectivity of enantioseparation. Isotope effect increased with increasing number of deuterium atoms in the molecule. Some difference between the retention of isotopomers was observed but this was not sufficient for their separation. Analysis of the vibrational frequencies and related zero-point vibrational energies calculated for AMP isotopologues by quantum mechanics allowed to disclose some pivotal factors contributing to retention and enantiorecognition, profiling a model based on the interplay between hydrogen bonding- and dispersion-type interactions. Number and location of the deuterium atoms in the AMP impact the strength of the pivotal interactions. Increasing deuteration degree, in particular at positions close to the amino group, was found to favor hydrogen bonding-type forces, whereas it resulted detrimental for dispersion forces.

NAA P-03

Retrospective data analysis for benzimidazole opioids and their metabolites in post-mortem and clinical urine samples

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Background & Aims: The emergence of benzimidazole opioids has gained increasing attention worldwide during recent years. Isotonitazene was first detected in Finland during a medicolegal investigation in 2021. Since then, etomethazene (5-methyl etodesnitazene) has been detected twice, protonitazene once, and metonitazene three times towards the end of 2023. However, in November 2023 the National Bureau of Investigation released a national alert about a seized batch of fake Subutex tablets containing metonitazene. Based on the alert, we decided to set up a targeted benzimidazole opioid database expanded with simulated and tentatively identified benzimidazole opioid metabolites, and to conduct a systematic retrospective data analysis to all post-mortem (PM) and clinical urine sample data acquired with ultra high performance liquid chromatography high resolution quadrupole time-of-flight mass spectrometry between August and November 2023. The aim was to further investigate whether any additional benzimidazole opioid positive cases were to be found with an expanded database.

Methods: A benzimidazole opioid database containing 21 parent compounds and 24 simulated or tentatively identified metabolites was created for TASQ (Bruker Daltonics GmbH, Bremen, Germany) software. The parent compound information was extracted from HighResNPS (https://highresnps.com/), and at least diethylaminoethyl side chain N-dealkylated simulated metabolite was created for each parent compound. The simulation was manual, and it was based on tentatively identified metabolite candidates earlier observed in true positive cases for metonitazene and etomethazene (5-methyl etodesnitazene). These observed metabolite candidates were added to the data base as well: N-de-ethylation, O-demethylation and a combined N-and O-dealkylation for metonitazene, and

N- and O-de-ethylation and N/O-de-ethylation combined with hydroxylation for etomethazene (5-methyl etodesnitazene). Certified reference material was available for 8 compounds.

Results & Discussion: A total of 1977 PM and 7793 clinical urine sample data was processed against the benzimidazole targeted data base. No additional benzimidazole opioid positive PM cases were found. However, in clinical case material, a total of four positive cases were observed: one etomethazene (5-methyl etodesnitazene) in August, and three metonitazene cases during October. Notably, the metonitazene cases had a match for the tentatively identified metabolites only. In subsequent PM cases, we observed that this pattern has been repeated: the urine gives a match for the metabolite only, and quantitative target analysis on blood confirms that metonitazene has been taken prior to death.

Conclusion: The results did not indicate a benzimidazole opioid poisoning epidemic, as only four additional positive cases were found in the retrospective data analysis of nearly 10 000 samples. The result emphasized the challenges of parent compound detection in urine samples. Having the tentatively identified metabolites included in the database enabled the detection of the additional metonitazene cases. This result points out the demand for alternative protocols for compound identification when reference material is not available.

HPLC-MS/MS stereoselective determination and quantification of MDMA and its phase-1 metabolites in human oral fluid samples: estimation of consumption time

NAA P-04

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Background & Aims: 3,4-Methylenedioxymethamphetamine (MDMA) represents one of the most abused drugs worldwide with about 20 million estimated users, with 2.820.000 of them just in Europe (WDR 2023). In humans, ecstasy-type substances improve and communication, stimulate positive mood changes, increase self-esteem, alertness, and induce euphoria. Many severe or even fatal intoxications were reported. Due to the broad spectrum of effects, a forensic assessment of current impairment is particularly relevant in cases of driving under the influence of drugs. MDMA contains an asymmetric carbon and exists as a racemic mixture. The S-(+)- isomer of MDMA has been reported to be a more potent neurotoxin than the R-(-)-isomer, which seems to exhibit more mescaline-like effects. Also, other pharmacological, toxic, and toxicokinetic properties of MDMA enantiomers are known to vary significantly. Estimating the time of consumption and the current influence of the stimulant is particularly difficult when only total MDMA concentrations are considered. This study aims to stereoselectively analyze MDMA and its metabolites to provide valuable information to facilitate the interpretation of the topic.

Methods: An enantioselective High-Performance Liquid Chromatography tandem mass spectrometry method for MDMA detection, and its major phase-1 metabolites 3,4-methylenedioxyamphetamine (MDA), 4-hydroxy-3-methoxyamphetamine (HMA), and 4-hydroxy-3-methoxymethamphetamine (HMMA) was developed. The method was fully validated according to OSAC guidelines (USA). It showed good selectivity, sensitivity, linearity, precision, and accuracy in intra- and inter-day tests. The method was applied to 46 oral fluid (OF) samples from roadside checks by law enforcement from Belgium and managed by the National Institute of Criminalistics and Criminology (Brussels, Belgium), and 104 OF samples from a randomized, cross-over, placebo-controlled, double-blind study conducted at the Hospital Universitari German Trias i Pujol, Institut d'Investigatiò en Ciènces de la Salut Germans Trias i Pujol (Badalona, Spain). 10 subjects (3 females and 7 males) were recruited and administered 100 mg MDMA. Approximately 0.5 mL of OF was collected with Salivette® tubes at baseline and 15, 30, 45 minutes, and 1, 1.5, 2, 3, 4, 6, 8, 10, and 24 hours after intake. The samples were processed by liquid-liquid extraction with chloroform: ethylacetate 9:1 (v/v) and injected in HPLC-MS/MS using polysaccharide-based chiral column Lux AMP 150×4.6 mm and a mobile phase containing 75% methanol and 25% (v/v) 5 mM ammonium bicarbonate buffer pH 11.0, in an overall run time of 8 minutes.

Results & Discussion: The highest concentration of both MDMA enantiomers was detected between 1.5 and 2 hours after intake, reaching 1210.3 ng/mL for 1st enantiomer and 1102,5 ng/mL for 2nd one. The MDMA 1st/2nd enantiomers ratio ranged from 1.1 to 5.3, and showed an increasing tendency over time, obtaining maximum values at 24 hours after intake. MDA was the metabolite found at higher concentrations than HMMA and HMA. Average MDA concentrations ranged from 2.6 to 12.9 ng/mL and 0.8 to 32 ng/mL for the 1st and 2nd enantiomers, respectively. In both cases, the highest concentrations were reached between 2 and 3 hours after consumption. The 1st/2nd enantiomers ratio for metabolites was also calculated. As MDMA, the trend of the ratio increased over time.

In the OF samples from the roadside checks, only MDA was identified as a phase-1 metabolite, and its enantiomers were quantified along with those of MDMA. Next, the 1st/2nd enantiomers ratios calculation for MDMA and MDA was applied. The first ranged from 0.9 to 5.4 (just one was 54.8), while the second was in the range of 0.19-2.6. Comparing the MDMA 1st/2nd enantiomers ratio with that of the clinical study samples, the values are within the range. Therefore, it is possible to assume that all subjects had taken MDMA from 15 minutes to 24 hours before sampling. In contrast, the outlier value (54.8) may refer to an intake that occurred more than 24 hours earlier. MDA concentration and its 1st/2nd enantiomers ratio also correlated with that of the clinical study samples.

Conclusion: OF samples from the clinical study and roadside controls were stereoselectively analyzed to quantify MDMA and its metabolites enantiomers, with the first enantioselective comprehensive HPLC-MS/MS method. OF allows an interpretation of real pharmacokinetic parameters useful in the investigation of drug metabolism, like the blood matrix. In contrast to blood, OF collection is rapid, non-invasive, and safe. However, if the drug is snorted or taken orally, in realistic settings, we must take contamination of OF shortly after intake into consideration. Data showed that the 1st/2nd enantiomers ratio correlates with MDMA metabolism, where lower values correspond to a short time since intake. The impact of the route of drug consumption should be evaluated in the future. Therefore, the determination of the absolute OF MDMA and its metabolites concentration, accompanied by the determination of the 1st/2nd enantiomers ratio of MDMA represents a useful and quick way of interpreting the time of consumption and thus the current influence of the stimulant.

NAA P-05

Quantification of street cocaine with NIR spectroscopy

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Background & Aims: The illicit drug trade, particularly cocaine, poses significant challenges to public health. Traditional analytical methods for street drugs analyses are often time consuming and require sample preparation. NIR spectroscopy offers a promising alternative by providing fast and accurate results without the need for extensive sample preparation.

Methods: A series of binary cocaine mixtures was prepared from cocaine hydrochloride and cutting agents levamisole hydrochloride, phenacetine, lidocaine, caffeine, creatine and paracetamol. A series of 22 street samples of cocaine were obtained from Czech Police and Czech Customs. Both series of samples were analysed by MicroNIR On-Site W and data were evaluated by NIRLab cloud software and a Camo Unscrambler X. Street samples were also analysed by UHPLC-MSMS Thermo ScientificTM Vanquish and Orbitrap ExplorisTM 120.

Results & Discussion: Chemometric techniques such as multivariate analysis were employed to develop calibration models for quantifying cocaine levels in unknown samples. These models were applied in a meassurement of 22 street samples of cocaine, which were also analysed by LC-MS. Preliminary results demonstrate the potential of NIR spectroscopy for quantifying street cocaine. The developed calibration models show promising results in identification and quantification of cocaine in unknown samples.

Conclusion: This study explores the feasibility of using NIR spectroscopy for the quantification of street cocaine. The findings from this study contribute to the growing body of research on the use of NIR spectroscopy in combating illicit drug trafficking and enhancing public safety. The advantages of NIR spectroscopy, including its speed, non-destructiveness, and cost-effectiveness, make it a valuable tool for rapid screening and quantification of illicit substances, which may have a potential to revolutionize drug analysis in forensic and law enforcement contexts.

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NAA P-06

Determination by UHPLC-MS/MS of 18 common drugs of abuse and metabolites, including THC and OH-THC, in volumetric Dried Blood Spots: method development and validation

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Background & Aims: Due to the increased consumption of traditional drugs of abuse and cannabis, forensic toxicology laboratories are increasingly called upon to develop new and effective methods. Nowadays, there is a need for easy and fast sampling techniques, which can be performed on-site (e.g. workplace drug or roadside

testing), and for simple, easily applicable but sensitive methods, which provide accurate results. This study aimed to develop and validate an analytical method for the simultaneous determination of 18 common drugs of abuse and their main active metabolites using the Dried Blood Spot (DBS) technique as microsampling method and the UHPLC – MS/MS for the samples analysis. Specifically, the detection panel consisted of 6-MAM, amphetamine, benzoylecgonine, buprenorphine, cocaethylene, cocaine, codeine, EDDP, MDA, MDMA, methadone, methamphetamine, morphine, norbuprenorphine, ketamine, norketamine. THC and its psychoactive metabolite, OH-THC, were also included.

Methods: The validation protocol was based on the threefold repetition of the calibration curve on three different days, that provides the advantage of allowing the evaluation of LOD, LOQ, intra and inter-day precision and accuracy, in addition to the calibration model. Specifically, blank whole blood was spiked with all 18 analytes at six concentrations (1, 5, 10, 25, 50 and 100 ng/mL) in three working sessions along five days. 10 µL were deposited on Capitainer®B DBS cards and left for 3 h to dry at room temperature. After liquid-liquid extraction with 500 µL of methanol added with 2.5 µL of a mix of deuterated internal standards, intense stirring and 30 min sonication followed. Each extract was then transferred into a fresh tube prior to evaporation under a nitrogen flow at room temperature. The dry residue was reconstituted in 30 µL of 5 mM formic acid in water: 5 mM formic acid in acetonitrile (50:50 v/v) and then injected into the SCIEX Triple QuadTM 7500 LC-MS/MS - QTRAP®, which allowed to reach higher levels of sensitivity and precision. Independent experiments were carried out for the evaluation of the recovery and the matrix effect, which were assessed at a low (2 ng/mL) and a higher (75 ng/mL) concentration level for all analytes. The data obtained for all the validation parameters were uploaded to the Methods Validation App, a free online platform, aimed to provide a systematic workflow, organized across multiple pages, for the validation of analytical methods. Stability experiments for all analytes tested in different storage days and temperatures were also included.

Results & Discussion: The calibration curve for each analyte was created by calculating the peak-area ratios between the target analyte and the respective ISTD for all concentration levels. The selection of the best calibration model was carried out based on the Levene's test for assessing heteroscedasticity, the weight factor was selected using the evaluation of the lowest normalized total variance score and the Mandel's test was chosen for model order selection. Linearity was achieved in the range of 1-100 ng/mL for all analytes, except from norbuprenorphine that was linear in the range of 5-100 ng/mL. The LOD was computed using the Hubaux-Vos method and ranged between 0.5 ng/mL (cocaine) up to 1.8 ng/mL (cocaethylene and OH-THC), with only norbuprenorphine having a LOD of 3.2 ng/mL. LOQs were considered as twice the LODs. Replicates from each day and the entire validation set allowed for the calculation of intra and inter- day precision (CV%) and accuracy. The average CV% was within 20% for all compounds with few exceptions concerning the inter-day precision and the average bias for intra and inter- day accuracy was within 10%. Average recovery at low and high concentration levels was at 60% and 66%, respectively, while average matrix effect was found to be at 100% and 116% respectively. Codeine, MDA, MAMP and OH-THC were omitted from this calculation as they showed a matrix effect higher than the accepted range. The method was applied to previously analyzed authentic blood samples which were spotted onto the DBS card. The presence of drugs of abuse (e.g. cocaine, ketamine, and THC) was confirmed (minimum value detected: 1.3 ng/mL).

Conclusion: This method proved that a 10 µL dried blood sample can be an effective micro sampling approach for drugs screening protocols while remains highly sustainable as low sample volume, less extraction solvent and less time is needed to obtain accurate results. The use of UHPLC-MS/MS demonstrated its significant capability to quantify the target analytes with high reliability at low concentrations in small specimen volumes and all the validation parameters showed that the method is reliable and is ready to be used in a daily workflow. This procedure improves the efficiency of toxicological testing and provides an advance for identifying the ongoing illicit compounds trade.

Nuclear magnetic resonance (NMR) techniques for the determination of benzylone in urine

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NAA P–07

Background & Aims: Nuclear magnetic resonance spectroscopy (NMR) is an analytical technique used mainly to provide structural elucidation and characterization of molecules in pure form or in mixtures. Herein, various NMR techniques were applied to determine the molecular structure of benzylone (3,4-methylenedioxy-N-benzyl cathinone, BMDP) and quantify it in human urine.

Methods: NMR spectra were acquired on a Bruker AVII 500 MHz spectrometer operating at 500.13 MHz equipped with either an inverse triple resonance probe (TXI) with a shielded Z-gradient and on a Bruker NEO spectrometer with a cryoprobe (NEO-TCPI) (Bruker Biospin, Rheinstetten, Germany). Experiments were performed at 298°K. The processing of spectra was implemented using the Topspin 4.2 software. 2D 1H-1H COSY, 1H-1H TOCSY, 1H-1H NOESY, 1H-1H ROESY, 1H-13C HSQC, 1H-13C HSQC with WET solvent suppression with shape pulse, 1H-13C HMBC

NMR experiments were performed using standard Bruker software and parameters were optimized. TSP-d4 was added as reference standard (both for quantitation and frequency calibration). Spiked urine samples with benzylone were measured with qNMR in respect to the standard TSP-d4 solution to calculate the LOD.

Results & Discussion: 1D and 2D NMR experiments were performed and the assignment of the 1H and 13C chemical shifts is provided. Additionally, the contribution of possible exchange phenomena to the spectrum pattern of benzylone and the concentration effect on its chemical shifts is reported. Additionally, diffusion ordered spectroscopy was applied in order to measure its diffusion value in an aprotic solvent (DMSO-d6). The Nuclear Overhauser Effect (NOE) constrains have been studied by performing NOESY and ROESY experiments.

The HSQC-TOCSY experiment showed that the cross-peak of H11-C11 to H14-C14 is characteristic for the benzylone fragment moiety. Along with the existence of the H-8 proton, they are a strong indication of the existence of a "benzylone-like" moiety.

Chemical structure assignment of benzylone was also performed in spiked urine samples. The addition of a specific volume of DMSO-d6 to the spiked urine sample eliminated the residual water signal revealing the overlapped H11 and H8 benzylone peaks.

Sensitivity was expressed by the limit of detection (LOD) and limit of quantification (LOQ) for the 1H NMR, defined as the lowest concentration giving a signal-to-noise ratio of three (S/N = 3) and ten (S/N = 10), respectively. The LOD value for benzylone was found to be 0.25 µg/mL.

Conclusion: The unidimensional and bidimensional 1H and 13C NMR data were completely assigned, allowing the determination of the molecular structure of benzylone. Also, it was observed a "NMR spectroscopic pattern/signature" possibly indicating a "benzylone-like" moiety in an unknown sample.

Overall, NMR spectroscopy, bearing its inhering advantages, could be an essential tool in the ongoing effort to understand and address the challenges posed by NPS detection in biological samples.

NAA P-08

Prediction of street-level heroin potency from analysis of cigarette filters or other paraphernalia

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Background & Aims: In recent years, fatal and non-fatal heroin-related overdoses have increased in north-eastern Italy, and the change in potency of heroin available at street level has been identified as a prominent factor associated with acute toxicity. Two very different products, high-potency and low-potency heroin were becoming available on the street, and no clear morphological characteristics could be used to easily distinguish them. A theoretical model for predicting potency of street-level heroin from rapid analysis of cigarette filters and other paraphernalia was developed and experimentally validated as part of an overdose prevention project.

Methods: First, a laboratory procedure was developed to produce test filters according to the steps of heroin injection, starting with different amounts of heroin powder and testing different types of filters by excluding or including pH-regulation steps and by trying different extraction solvents. The procedure was then applied to both test and real filters used in the preparation of heroin injections. Areas of the chromatographic peaks of diacetylmorphine (heroin), 6-monoacetylmorphine (6-MAM) and caffeine, obtained from GC-FID analysis of organic extracts of filters were used to develop the model. Caffeine, which is consistently present and systematically related to heroin content in real heroin samples, possibly due to a recurrent dilution process, was used as an internal standard to predict heroin potency, defined as the weighted sum of heroin and 6-MAM content. The robustness and predictive efficiency of the model was investigated over a wide range of potencies on laboratory prepared filters and real filters used to prepare heroin injections. GC-MS and LC-HRAM-Orbitrap-MS analyses were also used to identify other psychoactive substances that may be present in low concentrations in real filters, such as new synthetic opioids (NSOs, e.g. fentanyl analogs or non-fentanyl NSOs) or other new psychoactive substances (NPS). Cigarette filters were used as the standard material, but other materials (e.g. spoons, syringes, wrappings) were also tested. The model was also tested in two recent fatal overdose cases where both heroin powder samples and paraphernalia were available.

Results & Discussion: Quantitative analysis of a filter as a whole would not provide significant information about the concentration of active ingredients in the original powder used to prepare the injection. The developed model

overcomes this problem by using ratios of area peak values of heroin, 6-MAM and caffeine obtained from filter analysis to predict heroin potency. This is feasible because the three drugs are consistently present in heroin samples and caffeine is systematically related to heroin, so it can be used as an internal standard. The procedure for the preparation and analysis of the filters is fast and feasible in the laboratory (total time about 15 min). To develop the model, a new experimental parameter (rP) was first defined as the ratio between potency and caffeine concentration. Then, rP values were obtained from 70 real heroin samples and a model equation was determined that was able to predict potency from rP. The theoretical implications of the model equations are reported and discussed. The model was applied to laboratory prepared and real heroin samples and showed strong predictive ability. The relative standard deviation associated with the predicted potencies was always less than 15%. By applying a coverage factor (k=2), the recoveries associated with the prediction bands were mostly in the range of 80-120%. Extremely adulterated heroin samples gave the largest relative analytical errors. The model was used to implement a rapid alert system to inform drug users and health facilities about the potency of heroin or NSOs and/or other NPS circulating in the area.

Conclusion: The developed model for predicting the potency of street heroin from the analysis of filters used to prepare injections is a dynamic tool whose parameters can be updated according to market characteristics, so that it can be useful for laboratories involved in drug analysis and prevention programmes. For example, filters used to prepare heroin on the street could be collected on a regular basis and then analysed to provide a picture of trends in the heroin market, or the tool could be used as part of the forensic analysis of overdose cases to infer potency when only paraphernalia is available.

Do isotopologues have always the same retention time in achiral and chiral chromatography?

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Background & Aims: The separation of isotopologues resulting from the isotopic labelling of molecules with deuterium (D, 2H) became especially important in the past few decades. The reason for this is a steadily increasing realization of the importance of well-known kinetic isotopic effects in biological systems. Since the activation energy of the C-D bond is higher compared to the C-H bond, the compounds containing deuterium instead of hydrogen (protium) undergo slower transformations in the living body, as well as in the environment. This, in principle, may lead to favorable metabolic and pharmacokinetic properties and lower toxicity of deuterated biologically active compounds. Prolonged stability of compounds is of interest not only with medicines but quite important also with other types of compounds such as agrochemicals, chemicals used in the electronic industry, etc. Thus, separation of isotopically labelled (especially deuterated) compounds is becoming a hot topic in separation science. Isotopologues have quite similar physico-chemical properties that makes their chromatographic separation challenging.

In the present study the separation of isotopologues with several achiral columns as well as the simultaneous separation of enantiomers and isotopologues of some chiral amphetamine derivatives with polysaccharide-based chiral columns were attempted in HPLC.

Methods: A HPLC 1290 Infinity II (Agilent Technologies Italia S.p.a., Milan, Italy) instrument coupled with a mass spectrometer (6470A Triple Quadrupole LC-MS) equipped with an electrospray ionization source (ESI) operating in both positive and negative mode was used. Data were acquired with MassHunter® Workstation Qualitative Analysis 10.0 Software (Agilent). Analysis of all compounds (3,4-methylendioxymethamphetamine (MDMA), 3,4-methylendioxyethamphetamine (MDEA), 3,4-methylendioxyamphetamine (MDA) and methamphetamine (MET)) were performed on two chiral (Lux- AMP and Lux Cellulose-3) and three achiral (Kinetex 2.6 µm Phenyl-Hexyl, Kinetex 2.6 µm Biphenyl and Luna Omega 1.6 µm Polar C18) columns.

Results & Discussion: There was only a very limited isotope effect with achiral columns operated in methanol containing mobile phase. By limited effect we mean limited separation between the analyte of interest and its deuterated isotopologue. With addition of ammonium bicarbonate buffer a "normal" but still weak isotope effect was observed with all columns for all 4 studied compounds. The extent of the effect decreased with increasing the mobile phase buffer content and, in some cases, turned into an "inverse" isotope effect at higher buffer content. Since all analytes of interest to this study were chiral, additional complexity in their analysis was expected with chiral columns. No separation of enantiomers was observed for any of the studied compounds on Lux Cellulose-3 with

methanol or methanol-ammonium bicarbonate mixtures as mobile phases. With the Lux AMP column in combination with methanol or aqueous methanol as mobile phase, both successful separation of enantiomers, and the so called "inverse" isotope effect was observed for all 4 studied compounds. On the same chiral column enantioseparation along with "normal" isotope effect was observed for all 4 studied compounds with ACN modified with 0.1 % (v/v) aqueous ammonium hydroxide and low content of aqueous phase. This effect from the viewpoint of separation of isotopologues is opposite to that observed on the same column with methanol containing 0.1 % (v/v) ammonium hydroxide. With increasing content of the aqueous phase this effect decreased similarly to the observations on achiral columns and at a certain content of the aqueous phase the isotope effect disappeared. After further increasing the content of aqueous component the isotope effect re-emerged but the elution order of the isotopologues reversed. Thus, based on the mobile phase composition, it was possible to control intermolecular noncovalent selector-selectand interactions in chromatography and switch from the "normal" to the "inverse" isotope effect.

Conclusion: A strong isotope effect was observed on achiral and chiral columns for some amphetamine derivatives in high-performance liquid chromatography under the applied LC conditions. On achiral columns heavier (deuterated) compounds were retained longer compared to their non-deuterated analogues and thus "normal" isotope effect was observed in ACN and aqueous ACN while in methanol and aqueous methanol the effect was mostly limited. On the chiral column Lux AMP the "inverse" isotope effect was observed in methanol and aqueous methanol, while on the same column in ACN and low content of aqueous component in the mobile phase a "normal" isotope effect was observed. This effect decreased with increasing content of the aqueous component in the mobile phase. It was demonstrated that based on the content of aqueous component in the mobile phase it was possible to switch from "normal" to "inverse" isotope effect. Some correlations between the separation of enantiomers and the separation of isotopologues were observed for methamphetamine. Preliminary calculation results supported the hypothesis that polar hydrogen bonding-type noncovalent interactions are responsible for the "normal" isotope effect while "inverse" isotope effect is mostly caused by nonpolar/hydrophobic selector-selectand interactions and apparently also dispersive interactions.

NAA P-10

Simultaneous separation of 2, 3 and 4 CMC and their enantiomers using HPLC-MS/MS method. Application to real human cases.

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Background & Aims: Identification and simultaneous separation of positional isomers and their enantiomers is quite challenging in forensic toxicology, particularly in the context of new psychoactive substances (NPS). Despite the very similar chemical structure, positional isomers as well as their enantiomers often show different properties in terms of metabolism, pharmacokinetics and pharmacodynamics and thus can exhibit dramatic differences with respect to their toxicity. Additionally, besides these pharmacological and toxicological effects, the legal status is also of great importance. We present a sensitive and selective UHPLC–MS/MS method able to simultaneously separate the ortho, meta and para isomers of chloromethcathinones (CMC) and their enantiomers using Lux AMP chiral column. The method was also applied to positive oral fluid (OF) samples in order to demonstrate its applicability to real cases and its ability to discriminate even their major metabolites.

Methods: Separation was performed on a HPLC 1290 Infinity II coupled to a mass spectrometer (6470 A Triple Quadrupole LC-MS) equipped with a jet stream electrospray ionization source operating in positive mode. After initial screening of five polysaccharide-based chiral columns in combination of different mobile phases, ratio and flow rate, the simultaneous separation and determination of CMC's positional isomers and their enantiomers was performed using amylose-based chiral column Lux AMP (15 cm length); methanol and 5 mM ammonium bicarbonate (pH=11.5, adjusted with ammonium hydroxide aqueous solution) in water were used as mobile phase in the ratio of 80/20 (v/v). Flow rate of mobile phases was 0.75 ml/min. Method was validated following recommendations of the Organization of Scientific Area Committees (OSAC) for Forensic Science. Real human OF samples were firstly screened using high resolution mass spectrometer (Thermo Scientifc Q Exactive mass spectrometer). The 9 real OF samples positive for 3 and/or 4 CMC were prepared using following procedure: 10 µL of internal standard (mephedrone-d3) and 250 µL of acidified ACN (0.2% HCl) were added to 100 µL of sample and mixed well. After centrifugation supernatant was collected and evaporated under nitrogen flow, then residue were reconstituted with 100 µL mobile phase and 5 µL was injected in the chromatographic system.

Results & Discussion: Lux AMP (15 cm length) was chosen as chiral column, in combination with 80% (v/v) of methanol and 20 % (v/v) of 5 mM aqueous ammonium bicarbonate buffer at pH 11.5 as mobile phase. This UHPLC-MS/

MS method was useful to easily detect and quantify all CMC's positional isomers as well as their enantiomers, in a total chromatographic run time of 12 minutes. A full separation of 3- and 4-CMC enantiomers but partial separation of 2-CMC enantiomers was obtained. The method was subsequently applied to 9 real OF samples positive for 3 and/or 4 CMC. Out of nine samples, two were positive for both 3 and 4-CMCs. None of them was positive for 2-CMC. The maximal concentration found for 3-CMC was 2,409 ng/mL for the first enantiomer and 2,697 ng/mL for the second. Regarding the two 4-CMC positive samples the highest concentrations were 92.1 and 104.4 ng/mL for the first and second enantiomer, respectively. The 3- and 4-CMC positive samples reported the highest concentrations was re-analysed for the identification of their main metabolites. It was possible to identify 4 metabolites (Metabolite-N1, -N2, -N4, and -N5) of 3-CMC out of 7 total. Out of 4 detected metabolites, enantiomers of 3 were fully separated, while for metabolite-N5, which has 2 chiral centres, just two peaks were detected. Since the concentrations of 4-CMC in these samples were much lower than 3-CMC and they share same MRM transitions, also the response of 4-CMC metabolites were lower in comparison to metabolites of 3-CMC, thus the response of its metabolites were lost in the baseline.

Conclusion: A fast, selective and powerful method was developed and validated for the simultaneous separation and quantitative determination of CMC positional isomers and their enantiomers. This method met the requirements of selectivity and linearity. Analytical limits, matrix effects and recovery were satisfied. The method was also found to be suitable for the determination of some of 3-CMC main metabolites.

Advancing benzodiazepine consumption monitoring: Insights from wastewater-based epidemiology

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Background & Aims: Benzodiazepines (BZDs), a class of psychoactive pharmaceuticals, are commonly prescribed for conditions such as anxiety and insomnia. Due to their widespread consumption and potential for dependence and misuse, they are interesting but challenging to monitor consumption in the population. Conventional methods of estimating benzodiazepine consumption, e.g., sales records, prescription data, and surveys, often suffer from limitations such as reporting biases, incomplete or no coverage, and substantial lag time before data becomes available.

Wastewater-based epidemiology (WBE) is based on the analysis of human metabolic excretion products of xenobiotics, including BZDs, in influent wastewater. This approach provides an objective, and near real-time estimation of BZD consumption at the population level. We aim to demonstrate the usefulness of WBE for assessing consumption of commonly prescribed BZDs, specifically to evaluate spatial trends and patterns in BZD consumption. Further, fundamental knowledge to correctly interpret the complex metabolic pathways linked with BZDs will be gathered, aiming to support future WBE monitoring campaigns. Through this, we aim to enhance our understanding of benzodiazepine consumption patterns and enable timely information to help interventions aimed at promoting safe and appropriate BZD use.

Methods: One consecutive week of daily influent wastewater samples were collected from different wastewater treatment plants in Belgium (Brussels), Latvia (Riga), Lithuania (Kaunas, Klaipeda, Vilnius), and Romania (Bucharest) (n=42) and analysed for a broad range of commonly dispensed prescription BZDs, z-drugs, and their human metabolites (n=27). Measured concentrations (ng/L) were back-calculated to population-normalised mass loads (PNML), a proxy for consumption, allowing for spatiotemporal comparison by considering the flow rate and population size.

Results & Discussion: In total, 14 different BZDs (and metabolites) were quantified at least once in a location. The compounds alprazolam, clonazepam (through metabolite 7-aminoclonazepam), diazepam, lorazepam, nordazepam, zolpidem, and zopiclone were quantified at least once (range: 0.03 to 24.58 mg/day/1000 people) in each locations. Intra- and intercountry differences in the quantified compounds and consumption levels were found. For alprazolam, clonazepam, zolpidem, and zopiclone measured PNMLs loads can be directly compared between the countries. For example, highest consumption of zolpidem was observed in Brussels (1.63 mg/day/1000 people), followed by Vilnius (0.91 mg/day/1000 people). The highest consumption of zopiclone was noted in Riga (6.42 mg/day/1000 people), while Brussels (0.82 mg/day/1000 people) exhibited the lowest consumption amongst the measured regions.

However, critical evaluation of the PNML of some biomarkers is required due to the complex overlap in metabolic pathways and, in some cases, absence of compound-specific biomarkers. For example, while oxazepam is sold as a parent drug, it is also a metabolite of diazepam, temazepam, and nordazepam. Therefore, temporal changes may be due to changes in BZD consumption (more/less), changes in the specific BZD consumed, or both. Spatial comparisons are further complicated by differences in regional prescription patterns and drug availability. For

example, oxazepam is marketed in all countries except Romania, whereas nordazepam is only sold in Belgium. Nevertheless, the measurement of compound- and class-specific BZD biomarkers in IWW can highlight spatial differences. Within this frame, temazepam was identified as a potential marker for diazepam use since it is not sold as a parent drug in aforementioned countries.

Conclusion: Wastewater-based epidemiology provides a framework for assessing BZD consumption patterns across diverse regions and time periods. This research gathered fundamental insights on the presence of BZD biomarkers in influent wastewater, but further highlights the complexity inherent in interpreting spatiotemporal trends due to overlapping metabolization pathways, regional variations in prescription patterns, and drug availability. This better understanding will advance WBE as an effective epidemiological tool for informed decision-making and the implementation of effective intervention strategies.

NAA P-12

Design and assessment of a microsampling-based interlaboratory comparison for phosphatidylethanol analysis

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Background & Aims: Phosphatidylethanol (PEth) has emerged as a crucial marker for monitoring abstinence and assessing drinking behavior. Despite the increasing utilization of the alcohol biomarker, there remains a scarcity of standardization and comprehensive understanding regarding the robustness and comparability of the employed methods. In 2022 the first consensus for the use of the alcohol biomarker phosphatidylethanol was established (2022 Consensus of Basel), and to enhance the foundation for harmonization through accumulated experience, three rounds of interlaboratory comparisons involving authentic blood samples were conducted in the years 2022 and 2023.

Methods: Participating laboratories have been invited to send their dried blood spot (DBS) sampling devices to a central laboratory. Subsequently, authentic fresh blood samples derived from routine forensic and clinical cases were administered onto these sampling devices and returned to the respective laboratories. For each round, four samples with concentrations covering the decision limits according to the 2022 Consensus of Basel were used. The results were evaluated and compared by the central laboratory according to standards proposed by Horwitz and the Society of Toxicological and Forensic Chemistry (GTFCh). The most abundant analogue, PEth 16:0/18:1 was analysed and reported. The concentrations tested ranged from 16 to 480 ng/mL.

Results & Discussion: Five different microsampling devices and five different reference material manufacturers were used by the participants. Most of the laboratories used DBS card-based devices, followed by Capitainer®, Mitra® VAMS, and HemaXis devices. Analysis of the results according to Horwitz revealed that more than 85% of all values were within the acceptance criteria for successful participation ($|z\text{-score}| < 2$): In the first round, 16 laboratories participated with an overall success rate of 97%. In the second round, 19 laboratories participated with an overall success rate of 86%. In the third round, 20 laboratories participated with an overall success rate of 89%.

Conclusion: Interlaboratory comparisons based on microsampling are feasible without emphasizing a specific sampling device. For PEth, there is good comparability of the results in a large concentration range covering both lower and upper decision limit for most laboratories. However, there are still challenges in terms of calibration range and reporting limits.

NAA P-13

Fast and reliable quantification of cannabinoids by portable Near Infrared Spectroscopy

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Background & Aims: Analysis of cannabis flowers infused by semisynthetic cannabinoids is of growing interest due to its popular recreational use. The community of recreational cannabis users recently rediscovered the very rare natural cannabinoids (R/S)-Hexahydrocannabinol (HHC) as the demand for the legal alternatives of Δ9-THC. Cannabis flowers and edibles like gummi bears infused by HHC become very popular and cause a series of intoxications. Field cannabis testing by Near Infrared Spectroscopy (NIR) offers a non-destructive and rapid method for recognise a CBD, Δ9-THC or (R/S)-HHC infused cannabis flowers without the need for extensive sample preparation and chromatography analysis.

Methods: In this study, MicroNIR On-Site W was used to analyse a significant number of cannabis samples. Initially, the data was evaluated by NIRLab cloud software. To distinguish the HHC samples, we prepared the pure (R) and (S) enantiomers of HHC, which serve as analytical standards for the calibration curve and for the software training. The cannabis samples were also analysed by validated LC-MS method and portable GC-MS (Griffin 510). NIR model was build in Camo Unscrambler X based on the NIR spectra of the samples. The concentration of CBD, Δ9-THC and (R/S)-HHC obtained by GC-MS and CBD, CBDA, Δ 9-THC, Δ9-THCA and (R/S)-HHC obtained by LC-MS were used to fill the model data.

Results & Discussion: Quantification of cannabinoids was performed using GC-MS and LC-MS methods. The same samples were measured by NIR spectrometer. Based on the obtained NIR spectra and concentrations of cannabinoids within samples, multivariate analysis (PCA, PCR and PLSR) were employed to develop calibration models for quantification of CBD, Δ9-THC and (R/S)-HHC in unknown samples. We found slight difference with maximum error of 2 % of cannabinoids content between NIR and chromatography method, which is for preliminary results suitable in the field cannabis flowers quantification. The accuracy will be increased consequently with the total amount of measured samples used for further improve our calibration model.

Conclusion: This work presents a novel approach to quantifying cannabis flowers samples using NIR spectroscopy. The integration of NIR spectroscopy in forensic practise has the potential to enhance the ability for fast and reliable filed analysis, nevertheless the laboratory confirmation analysis by validated LC-MS and GC-MS techniques is further needed.

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Construction of a new turn-on fluorescent probe for the detection of hydrogen peroxide vapor

NAA P-14

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Background & Aims: With terrorist threats, environmental and health risks on the rise, sensitive and selective detection of explosives has become a global concern. As a result of their easy synthesis and high explosive power, peroxide-based explosives (PEs) have attracted widespread interest among the conventional explosives. H2O2 plays a dual role in PEs: not only as the starting synthetic material, but also as the decomposition product of the PEs, making it a signature compound for detecting PEs. In particular, achieving highly selective and sensitive detection of vapor emanated from explosives is a significant practical application for explosives monitoring. Therefore, we aim to develop a rapid and accurate method for H2O2 vapor by constructing a new turn-on fluorescent probe based on benzil moiety with BODIPY derivative.

Methods: The proposed new turn-on fluorescent probe was designed by employing benzil as H2O2-recognition group and BODIPY as fluorophore for the detection of H2O2. By chemical conjugation of benzil group and BODIPY derivative, the constructed probe would show almost no fluorescence owing to fluorescence quenching via the intramolecular donor-excited photo-induced electron transfer (d-PET) process. However, in the presence of H2O2, the benzil moiety of the probe can be selectively oxidized by H2O2, resulting in the release of free BODIPY fluorophore (BOD-COOH) and a significant fluorescence increment. Thus, H2O2 could be detected by measuring the BODIPY fluorescence. As for the detection of H2O2 vapor, the probe solution was drop-coated onto a silica gel TLC plate with about 1.0 cm radius circle and subsequently dried for 10 min. Then the prepared probe-coated TLC plate in the saturated vapor of H2O2 generated in a 100 mL bottle, where approximately 20 mL of H2O2 solution (diluted down to various concentrations) was put in and sealed for 12 h to reach the equilibrium vapor pressure. After exposure to the vapor for different time intervals, the fluorescence images of the TLC plates were obtained on gel imaging system, achieving the rapid detection of H2O2 vapor.

Results & Discussion: Under optimum conditions, the proposed probe can detect H2O2 in linear detection ranged from 25 to 125 μM with a detection limit of 4.41 μM. Meanwhile, the designed probe shows good selectivity toward H2O2, which is not affected by other common reactive oxygen species (ROS) and ions from explosive residues. More importantly, the probe can be successfully applied for rapid detection (down to 20 min) of H2O2 vapor with good sensitivity (down to 7 ppb).

Conclusion: In summary, we have developed a new turn-on fluorescent probe to sensitively and specifically detect H2O2 vapor, providing great potential for real-time detection and monitoring of the PEs in the field of public safety, forensic analysis and environmental monitoring.

NAA P–15

Rapid quantitative screening of 16 synthetic cannabinoids in urine using direct analysis in real time-mass spectrometry (DART-MS) analysis

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Background & Aims: Immunoassay-based (IA) detection for drugs of abuse is commonly used as an initial screening step in urine-based drugs testing due to rapid generation of results and ease of automation. However, IAs suffer from significant issues with cross-reactivity leading to false positives, thus requiring costly and time-consuming chromatography-based confirmatory testing. As a cost-effective alternative, DART-MS provides quantitative and highly selective results greatly reducing or eliminating false positives compared to conventional IA-based drug screening. In this work, we report on the development of a rapid, chromatography-free screening approach for eighteen synthetic cannabinoids in urine: (4-cyano-CUMYL-BUTINACA (1), 4-fluoro ABUTINACA N-(4-hydroxybutyl) metabolite (2), 4-fluoro MDMB-BUTICA (3), 4-fluoro BUTICA butanoic acid metabolite (4), 4-fluoro MDMB-BUTINACA N-butanoic acid metabolite (5), 5-fluro ADB metabolite (6), 5-fluoro MDMB-PICA (7), 5-fluoro MDMB-PICA metabolite (8), ADB-4en-PINACA (9), MDMB-4en-pinaca butanoic acid metabolite (10), ADB-BINACA (11), ADB-BUTINACA (12), ADB-HEXINACA (13), AMP-4en-PINACA (14), JWH 018 N-pentanoic acid metabolite (15), MDMB-CHMICA metabolite (16)). This DART-MS screening method successfully measures the targeted synthetic cannabinoids in 96 samples at a rapid throughput of 23 seconds per sample.

Methods: For method development, triplicate calibration series were prepared by spiking certified drug-free urine with standards (0.1-2500 ng/mL) using deuterated AB-PINACA as an internal standard. Hydrolysis was performed by adding 50 µL Kura enzyme to 500 µL pre-spiked certified aliquots of drug-free urine and incubated at room temperature for 20 minutes. After hydrolysis, 500 µL 0.1 M Borax buffer (pH=10.4) and 2.5 mL 30:70 (ethyl acetate:n-chlorobutane) were added to each sample followed by a 30 second agitation. Samples were centrifuged at 4000 RPM for 10 minutes and the organic layer was transferred to glass vials and evaporated to dryness under N2 at 40°C followed by reconstitution in 100 µL MeOH. Reconstituted samples were vortexed for 30 seconds, and 2 µL aliquots of each sample were transferred onto a Bruker DART QuickStrip HTS-96 screen and allowed to dry under N2 gas at 40°C for 15 minutes. For analysis, the prepared QuickStrip-HTS 96 screen was loaded onto the automated XY transmission stage of a TQ-Plus (Bruker Daltonics) triple quadrupole mass spectrometer for DART-MS-MS analysis. Accuracy was determined in triplicate using certified drug-free urine without detectable levels of the 16 standards at 2 levels for each analyte within the linear range of each calibration series. Results were validated against LC-MS that was performed using 20 urine samples confirmed as positive for one or more analytes.

Results & Discussion: DART and TQ-plus parameters were optimized for high sensitivity, precision, reduced helium consumption, and fast analysis time. With DART gas temperature and grid voltage optimized at 300°C and 50 V, respectively, unique MS/MS transitions, collision energies, and MS scan times were successfully identified. DART-MS analysis of the synthetic cannabinoid panel resulted in good linear correlation of $R2 > 0.99$ for all measured analytes and a recovery between 89 and 110% for all 16 analytes across the defined calibration ranges. The reported lower level of quantitation (LLOQ) for all analytes is at or below common IA screening cutoff values of between 0.1 to 5 ng/mL for the synthetic cannabinoid panel. In a representative cross-validation plot of urine samples (n=20) the butanoic acid metabolite of MDMB 4-en PINACA concentrations ranged from undetectable to 362 ng/mL with a mean value of 170.5 ng/mL ± 15.2. Measurements using The newly developed DART-MS method were well correlated with LC-MS measurements ($R2=0.995$, $p<0.05$) with a slope of near unity at 0.95. The results in this work indicate that this rapid chromatography-free workflow using DART-MS for quantitative screening is sufficient at detecting all 16 analytes at or below the common cutoff values without the high rate of false positives associated with IA based screening approaches.

Conclusion: The validated workflow presented demonstrates the suitability of the DART-MS workflow as a rapid, quantitative, and selective alternative to conventional IA-based urine screening for 16 common synthetic cannabinoids and their metabolites by offering a quantitative method with the benefits of minimizing false positives typically associated with IA based screening, avoiding costly and unnecessary chromatography-based confirmatory testing.

NAA P–16

Social algorithm method as an element of artificial intelligence in data integration in systematic toxicological analysis of blood

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Background & Aims: Technological development in the area of computational methods using artificial intelligence (AI) is definitely entering forensic toxicology laboratories. A manifestation of this phenomenon is the increasing use of software containing mechanisms based on, for example, neural networks and expert systems in the analysis

of data obtained during research on biological material. The Mass Profiler Professional of Agilent can be given as an example. By definition, artificial intelligence refers to systems that exhibit intelligent behaviour by analysing their environment and taking actions - with a degree of autonomy - to achieve specific goals. These methods develop in a three-pronged way. The oldest solutions are expert systems, and today they are solutions imitating the functioning of humans, and in particular the human brain. The least developed seem to be systems using social algorithms (swarm intelligence algorithms), i.e. imitating social functions, in particular human relations in a group (actually they refer to ants, bees, etc.). In the case of detecting a substance, it is often necessary to combine data from various sources, i.e. cooperation in a group, which is similar to cooperation in a community. Therefore, software connection and data exchange between the devices involved in identification and the analyst are important. Social media algorithms can be used for this purpose. As part of the functioning of the forensic laboratory, creating a community of autonomous programs analysing data (according to S. Lem; personoids) from various analytical systems while mutually using the generated data to the greatest extent possible without the participation of an analyst. The data generated is to be modified or refined on an ongoing basis, which is intended to increase the system's efficiency and its development. The analyst will obtain a reduced amount of data, pre-analyzed to discard the least reliable data. So, his task will be limited to only the most probable outcomes. This is the aim of the presented research.

Methods: Python, PHP and XML were used to prepare the software, i.e. scripting languages created for network applications. The main data sources are LC-QTOF and HPLC-DAD techniques. Data obtained while using other techniques (e.g. GC-MS) are entered at this stage by the analyst into the databases available for verification/confirmation of the result and training/correction of previously collected data. For the purposes of data export, the software capabilities of a given instrument are used; data is usually exported to text formats, e.g. .csv or .txt. The integration also used NIST libraries available on the Internet.

Results & Discussion: Python language was used, which allowed for the determination of a function correlating retention times and UV spectra for use by other elements of the community (personoids). The entries in the database were linked to the results obtained by LC-QTOF. The names of potential xenobiotics, via the CAS number, were linked to the external NIST database, which facilitated the evaluation of the obtained results. This made it possible to obtain a description of the xenobiotic with physicochemical characteristics. These activities led to greater data transparency (LC-QTOF), their mutual verification and shortening the time of data analysis. The approach used reduced the number of potential xenobiotics required for verification by an analyst by more than tenfold comparing to traditional work. An additional functionality of the personoid community is to generate research reports thanks to connection with database implemented in the laboratory, also based on web applications.

Conclusion: The implementation of integrated software enabling communication between instruments and the operator (analyst) at the level of generated data significantly speeds up the process of obtaining a verified analysis result, and thus contributes to confirming its validity. The sense of this type of activities appears in situations where many instruments are used that generate results that require confrontation. Giving a human dimension to software is not unusual, because it is not uncommon for programs to have "human" names, e.g. Aida, Alexa, Nero, Ralph. Combining the possibilities of their activities multiplies their total potential.

Illicit drugs in wastewater: A new epidemiological tool for assessing consumption in Algeria

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Background & Aims: The collection of information on the prevalence of illicit drug consumption generally relies on various methods, such as face-to-face surveys, telephone interviews, self-administered questionnaires (paper or electronic), and the analysis of biological samples such as urine, hair, and blood. An innovative approach complementing these traditional methods involves the analysis of wastewater, allowing for the detection of drug residues. A pilot study in Algeria has been initiated, focusing on the analysis of wastewater from three sewage treatment plants in the wilaya of Algiers

Methods: Conducted between March 21, 2023, and May 31, 2023, this study aims to provide an overview of the profile of illicit drug and pharmaceutical consumption at the city of Algiers. Samples were collected from three distinct stages, following a wastewater mapping of the city of Algiers.
The wastewater was filtered, and internal standards (100 ng/ml) were added along with 2 mmol/L ammonium formate and 0.02% formic acid. For instrumental analysis via HPLC-MS/MS, the mobile phase consisted of 5mM formic acid as aqueous phase A and acetonitrile. A C18 column (100 × 2.1 mm, 1.7 µm) was used in gradient mode.

NAA P-17

Results & Discussion: Some substances showed 100% detection, suggesting a systematic presence « Benzoylecgonine, Codeine, MDMA, Carbamazepine, Pregabalin, Tramadol, Lamotrigine, and Metformin » and other substances have demonstrated high detection rates, varying between 80% and 100%. Morphine, Paracetamol. Pregabalin exhibits an exceptionally high average concentration of 40.9 µg/L, while Metformin, commonly prescribed for diabetes, and Paracetamol, a widely used medication, show concentrations of 14.9 µg/L and 11.2 µg/L, respectively.

The statistical analysis conducted to assess the weekend effect showed that there was no significant effect for certain substances (Benzoylecgonine, Morphine, EDDP, MDMA, and Carbamazepine,...) while it was significant for others (Codeine, Paracetamol, Amitriptyline, Citalopram,...) indicating varying usage patterns over time.

The evaluation of treatment plant efficacy through the comparison of mean substance concentrations at inlet and outlet points has advanced the comprehension of substance elimination mechanisms, emphasizing the important function of treatment infrastructure in environmental conservation.

Conclusion: The examination of illicit drugs in wastewater emerges as a promising approach for monitoring drug consumption at the community level. This type of investigation provides insight into the drug consumption profile within specific regions and timeframes, giving valuable data for health and safety management strategies, as well as proposals for preventive measures and interventions addressing this concern.

NAA P–18

Analyzing blood lead levels and isotopic ratios using ICP-MS: Exploring potential exposure sources

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Background & Aims: Lead is classified by the World Health Organization (WHO) as one of the 10 chemicals posing significant threats to public health. Preventive measures vary depending on the implicated source, with recommendations tailored to identified risk factors. There is a marked interest in the development of new approaches to identify lead sources, especially at lower doses. Currently, the identification of exposure sources relies on observing lead concentrations in the environment, such as paint, dust, soil, and water. In addition to these standard approaches, new techniques such as lead isotopic ratios (LIR) could play a valuable role in routine environmental investigations.The objective of this study is to investigate lead isotopic ratios in blood samples, as well as samples of paint, dust, and tap water, to identify the origin of elevated or moderate blood lead levels.

Methods: In this study, 65 volunteers were enlisted, chosen from diverse demographic backgrounds, including various age groups and genders, with the exclusion of those with occupational exposure to lead. In this investigation, our primary objective is to ascertain blood lead levels and assess the lead content in diverse environmental samples (Water, Paint, and Dust) utilizing inductively coupled mass spectrometry (ICP-MS). The evaluation of compatibility between blood and potential sources of overexposure is conducted through the meticulous comparison of Isotopic Ratios (IR) derived from the four lead isotopes: Pb204, Pb206, Pb207, and Pb208

Results & Discussion: The distribution of blood lead levels in our study cohort exhibits a range from 2.7µg/L to 77.8µg/L, with an average of 25.9µg/L, showcasing a male predominance. Regarding environmental samples, the mean lead content is determined to be 3.4µg/L for water, 38.3µg/m² for dust, and 2314.4 for paint.
IRs complement lead concentrations in various sources to identify potential overexposure sources, prioritizing informative ratios like 207/206, 206/204, and 208/204. Involving eight participants, the isotopic analysis helps exclude certain sources initially flagged for potential overexposure, such as household dust, as seen in individual 13-01. Spatial discrepancies between blood and dust IRs indicate distinct sources, while congruent IRs between paint and dust suggest a shared source, as observed in individual 04-01. The absence of a single exposure source is apparent, exemplified by individual 05-03, suggesting multifactorial influences such as lifestyle, diet, and environmental factors like leaded gasoline emissions.

Conclusion: Lead Isotope Ratios offer promise in identifying potential lead exposure sources such as water, dust, and paint, though further research with larger samples is needed for definitive conclusions. Integrating isotopic analysis into routine investigations can aid in discarding improbable exposure sources, preventing unnecessary remediation efforts, and guiding targeted preventive measures to mitigate environmental lead poisoning, particularly in vulnerable populations. The systematic application of LIRs, complemented by statistical analyses, provides a pathway to establish action levels and intervention procedures aimed at reducing blood lead levels and addressing environmental lead contamination effectively.

Poster gallery - SoHT F-P-1 to P-25

10:00 - 10:30 Wednesday, 4th September, 2024

SOHT F-P-01

Revealing polydrug abuse in ketamine users: Insight from hair analysis on drug consumption patterns

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Background & Aims: Ketamine, originally formulated as an anesthetic in the 1960s, has gained notoriety as a recreational drug due to its dissociative effects. While ketamine abuse has been prevalent in various regions globally, its widespread use notably surged in Asia since the early 2000s, as highlighted in the UNODC World Drug Report 2023. In Singapore, ketamine became a drug of abuse in the late 1990s, leading to its control under the legislation in 1999. In recent years, ketamine abuse has become increasingly prevalent, often in combination with other illicit drugs. Hair is useful for determining the drug abuse history and patterns due to its long detection window compared to other biological matrices. To gain an insight into the ketamine abuse patterns, an evaluation study was conducted on the hair samples obtained from 185 individuals who were tested positive for ketamine as well as other illicit drugs between 2021 and 2023.

Methods: Hair samples were obtained from suspected ketamine drug users. Each hair sample underwent an initial decontamination procedure involving sequential washes with dichloromethane (2 x 5 mL), water (5 mL) and methanol (5 mL). Subsequently, about 20 mg of decontaminated hair was pulverized in acidified methanol, followed by sample clean-up using supported-liquid extraction (SLE) and derivatization using heptafluorobutyric anhydride (HFBA) and hexafluoroisopropanol (HFIP). Analysis of both the processed hair sample and solvent washes was conducted using a GC-MS/MS equipped with a HP-5MS capillary column, with multiple reaction monitoring (MRM) as the acquisition mode. Corresponding deuterated internal standards were included in the process for quantification.

Results & Discussion: In this study, 185 hair samples were found to be positive for ketamine, with the detected concentrations of ketamine range from 0.51 to 390.4 ng/mg (median: 5.41 ng/mg), while norketamine concentrations ranged from 0.2 to 34.5 ng/mg (median: 1.0 ng/mg). The concentration ratios of norketamine to ketamine was found to be in the range of 0.010 to 1.289 (median: 0.265).

Among the 185 ketamine users' hair, only 11% (n=21) were detected exclusively with ketamine and its metabolite (norketamine) while the remaining 89% (n=164) were found to contain other illicit drugs. 3,4-Methylenedioxymethamphetamine (MDMA) was detected in 154 ketamine users' hair, showing the highest detection rate of 83%, out of 185 ketamine users' hair, followed by methamphetamine (METH) at 18% (n=33), 11-nor-9-carboxy-tetrahydrocannabinol (THC-COOH) at 4.3% (n=8), cocaine at 4.3% (n=8), nimetazepam at 2.7% (n=5) and monoacetylmorphine (MAM) at 1.1% (n=2).

In terms of the patterns of multiple drug use, majority of ketamine positive hair (n=115) was found to contain only MDMA, in combination with ketamine. This was followed by combination of ketamine together with only MDMA and METH (n=21), and ketamine with METH alone (n=7). This indicates that ketamine was commonly abused in conjunction with these amphetamines-type stimulants (ATS) rather than non-amphetamines. This could be due to preference of the users on the effects by consuming both ketamine and ATS at the same time, or due to the availability of these drugs in the local illicit drug market. Other drug types in combinations of ketamine consumption, including cocaine, cannabis, heroin and nimetazepam, were detected, but they were in small numbers.

Segmental hair analysis also shows positive for ketamine as well as other drug types in more than one segment, indicating some were actually frequent users of ketamine and other illicit drugs over a period of 3 months or more.

Conclusion: A study on the multiple drug patterns was conducted by analyzing the hair samples obtained from suspected ketamine users. Most of the ketamine abusers were found to be polydrug users, who were concomitantly abusing other controlled drugs, particularly with MDMA and methamphetamine. Whether this was due to personal preference of the users of consuming both ketamine and ATS at the same time, or due to the availability of these drugs in the local illicit drug market would remain to be seen.

SOHT F-P-02

The detection of AEME in hair samples where thermal hair straighteners have been used

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Background & Aims: Anhydroecgonine methyl ester (AEME) is a thermal by-product produced when cocaine is heated, as such it is commonly used as a biomarker to identify 'crack' cocaine use. However, as AEME is formed by heating cocaine, there could be instances when AEME formation is not due to 'crack' cocaine use.

The aim of this study was to review the data obtained from hair samples that have been analysed for cocaine where the hair had been heated using a thermal hair straightener.

Methods: Data from samples analysed for cocaine from 01 January 2024 to 31 March 2024, where the donor had declared using thermal hair straighteners, was reviewed to determine if there was any correlation between levels of AEME / Cocaine detected.

Results & Discussion: Of the 283 cases analysed by the laboratory between 01 January 2024 and 31 March 2024, 138 tested positive for cocaine. Of these 138 positive cases, 34 were also positive for AEME. Of these 34 cases, that were positive for AEME only 5 declared using 'crack' cocaine, 16 declared using cocaine but did not specify it was in the form of 'crack' and 13 did not declare using cocaine. However, caution should applied to the declaration as they are real cases - where the donor declaration may not reflect the actual use.

Interestingly, the majority of cases (104) that were positive for cocaine, did not have AEME detected where thermal hair straighteners had been used.

Conclusion: AEME is usually detected in samples where the cocaine levels are medium-high, and other metabolites are also detected. The majority of hair samples positive for cocaine that had heat applied via the use of thermal hair straighteners, did not have a positive AEME result.

Therefore, AEME remains a useful marker to determine the use of cocaine in the form of 'crack' cocaine. However, it is recommended that caution still be applied and the use of thermal hair straighteners always considered as 29 cases had AEME detected when 'crack' cocaine was not declared.

SOHT F-P-03

Forensic challenges: Distinguishing methamphetamine consumption from external contamination

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Background & Aims: Methamphetamine has emerged as the most abused drug in Singapore. Besides urine, hair has been used to detect drug use, particularly for determining history of drug consumption. However, interpreting hair testing results has been challenging due to the difficulty in distinguishing between active drug consumption and external contamination. To address this issue, an in-house study was conducted to simulate environmental contamination by artificially incorporation of methamphetamine in hair through exposure to sweat, smoke, and direct contact. The findings of the methamphetamine contamination study were then compared with results obtained from authentic hair samples from drug users and non-drug users (controls).

Methods: For sweat contamination, drug-free hair (n = 3) was sprayed with artificial sweat containing methamphetamine at either 'low' (6.5 ng/ml) or 'high' concentration (25.2 ng/ml), for 5 days, 5 times (2 mL each time) per day. In the smoke study, 2 mg of methamphetamine hydrochloride powder was heated and vaporised in an enclosed cardboard box containing the hair sample. In the direct contact study, 0.6 mg of 'Ice' was rubbed gently onto the hair for 1 minute.

All hair samples were subjected to decontamination procedure involving sequential wash using dichloromethane (2 x 5 mL), water (5 mL), and methanol (5 mL). Subsequently, all solvent washes and hair samples were analysed using GC-MS/MS and LC-MS/MS. The ratios of methamphetamine in the different washes to the methamphetamine in hair (wash to hair ratios) were computed and compared across the various profiles studied.

Results & Discussion: Neither methamphetamine nor its metabolite amphetamine was detected in the washes or hair samples of 10 non-drug users. Conversely, methamphetamine was detected in both the washes and hair samples collected from the methamphetamine contamination study and drug users.

The data collected from 40 drug users, comprising 82 data points, revealed that methamphetamine ratios in the combined dichloromethane wash to hair ranged from 0.0004 to 0.485, while water wash ratios spanned from 0.002 to 0.788 and methanol wash ratios ranged from 0.002 to 0.708.

In the methamphetamine contamination via sweat, the ratios of methamphetamine in the combined dichloromethane wash to hair ranged from 0.002 to 0.514, while water wash ratios ranged from 0.017 to 1.76 and methanol wash ratios ranged from 0.006 to 0.260. In hair exposed to methamphetamine smoke, the ratios of methamphetamine in the combined dichloromethane wash to hair ranged from 0.174 to 1.55, while water wash ratios spanned from 0.566 to 3.95 and methanol wash ratios ranged from 0.131 to 1.43. For direct contact with methamphetamine powder, the ratios of methamphetamine in the combined dichloromethane wash to hair ranged from 1.00 to 11.9, while water wash ratios ranged from 0.721 to 8.63 and methanol wash ratios ranged from 0.124 to 3.51.

The methamphetamine wash ratios from the sweat contamination study were similar to the drug users' hair. However, amounts of amphetamine detected (<0.05 ng/mg) were much lower than the amphetamine detected (0.189 to 10.8 ng/mg) in the drug users' hair.

The wash ratios from the smoke and direct contact with methamphetamine were found to be higher compared to those in the drug users' hair. The results suggest methamphetamine could be detected in the washes, particularly the dichloromethane wash when hair is exposed to smoke or through direct contact with methamphetamine powder.

Conclusion: Based on the current findings which includes data from authentic hair, there is potential to distinguish between active consumption and external contamination via smoke and direct contact by comparing their wash ratios and the amount of metabolite detected in hair

Application of hair analysis in pediatric post-mortem toxicology

SOHT F-P-04

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Background & Aims: Hair analysis offers retrospective insight into drug administration or exposure, making it an ideal specimen for pre-employment, workplace, or probation drug screening, as well as toxicology testing in child custody cases. Its noninvasive collection procedure, reduced risk of adulteration and substitution, and simple storage enhance its superiority. Moreover, hair analysis complements blood analysis due to its extended detection window, drug stability, and ability to detect parent drugs rather than just metabolites. Segmental hair analysis further allows for historical assessment of drug use/exposure and differentiates repetitive administrations from a single exposure. This presentation of case series will highlight hair as a valuable specimen in pediatric death investigations by providing crucial information regarding drug exposure patterns.

Methods: A comprehensive screening for alcohol and drugs was performed on whole blood using liquid chromatography-time of flight (LC-TOF). Confirmatory analyses were conducted using chromatographic techniques coupled with a mass spectrometric detector. Hair was initially examined to identify the proximal end, unless clearly documented, and then aligned. If present, follicles were carefully removed from hair strands to prevent contamination. Hair was subsequently segmented based on the date of interest or timeframe. Each hair segment (~20 mg for immunoassay screening or ~50 mg chromatographical analysis) underwent two rinses with methylene chloride (1 mL each) in a microcentrifuge tube to eliminate surface contamination. The second rinse was retained for analysis to examine the presence of significant external contamination. Decontaminated hair specimens were dried and ground using a bead beater. The hair grindings were then incubated in methanol (1 mL) for two hours at 45 °C. Drugs were screened using immunoassay, liquid chromatography-tandem mass spectrometry (LC-MS/MS) with scheduled multiple reaction monitoring (MRM), or gas chromatography-mass spectrometry (GC-MS) operating in full scan mode. Quetiapine was extracted from the hair grindings using a differential liquid-liquid extraction (LLE) technique, followed by derivatization using butyric anhydride, and analyzed by GC-MS in full scan mode. Fentanyl and methadone were extracted using LLE and analyzed using an LC-MS/MS equipped with electrospray ionization (ESI) operating in positive ion mode with MRM. Findings from the hair analyses were reported qualitatively.

Results & Discussion: In the case of a 10-month-old infant victim, a quetiapine concentration of 9500 ng/mL was reported in cardiac blood. The child resided with the father, who was prescribed the medication, for approximately three months before her death. A Seroquel® prescription bottle was found near the deceased. Hair analysis was conducted to explore the possibility of repeated administrations. Head hair was prepared and segmented to produce a 10 cm distal end segment for analysis. The hair segment tested positive for quetiapine, allowing the medical examiner to rule out an accidental single exposure by the infant. In another case involving a three-year-old child with a history of seizures, toxicological analysis of blood and hair segments excluded an accidental acute exposure to fentanyl from a single use, indicating ongoing exposure. The blood and hair segments (both the proximal and the distal ends) tested positive for fentanyl. The fentanyl concentration in chest blood was 51 ng/mL. As part of the death investigation, hair analysis of the mother was also conducted and showed the presence of fentanyl, cocaine, and benzoylecgonine. Lastly, the complementarity of blood and hair analyses was demonstrated in the fatality of a 15-month-old child. Analysis of inferior vena cava blood revealed methadone and EDDP at concentrations of 570 and 130 ng/mL, respectively. Based on the suspicious circumstances and the possibility of in utero exposure, segmental analysis was conducted. The presence of methadone in the proximal, middle, and distal segments confirmed repetitive exposures to drugs over time.

Conclusion: In the reported cases, toxicological analyses, including blood and hair tests, were instrumental in determining the causes of death and potential substance exposures in infants and young children. Segmental hair analysis provides a chronological profile of drug exposure, although the practice of collecting hair in post-mor-

tem examinations is uncommon. Nonetheless, hair analysis in conjunction with blood analysis presents complementary toxicology insights, proving particularly valuable in pediatric death investigations for establishing an accurate timeline of drug exposure. Despite potential limitations in interpreting hair results and concerns regarding external contamination, these case studies advocate for the collection of hair in addition to routine specimens such as blood, urine, liver tissues, and gastric fluid during autopsies, especially for pediatric toxicological examinations.

SOHT F-P-05

Cerumen as an alternate bio-matrix for drugs of abuse: correlation of analysis results with those for urine, blood and hair

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Background & Aims: In clinical and forensic toxicology, screening for drugs of abuse is usually considered as the first step. Generally, the standard operating procedure for testing of drugs in toxicological analyses consists of an immunoassay screening performed on urine, followed by gas chromatographic–mass spectrometric confirmation. Blood and urine specimens have some limitations, because these lack convenience of collection in some cases and the short half-life of drugs either in urine or blood, resulting in missing administered drug(s) after a few days. Different biological matrices have been proposed as alternatives to urine and blood in order to prove the presence of illicit drugs: principally, sweat, saliva, hair, bones and nails. Despite of their expanded use, hair and nails have some disadvantages, such as risk of external contamination, which was considered an issue making interpretation of results a challenge; in the case of a sweat patch, it must be worn for 3–7 days with a minimum of 48 h time to collect adequate sweat for analysis. Bones can only be used for postmortem analysis of drugs.

The present study aims to introduce 'cerumen' commonly Known as 'earwax' as an alternate matrix for biomonitoring of illicit drugs. In addition to its advantage as a non-invasive sampling technique, earwax could even be more preferred as a diagnostic biological secretion, because it is relatively less contaminated by the ambient air or by cosmetics that are the problems normally faced in the cases of sweat and hair.

Methods: The study included 250 participants aged 20–40 years. The study requirements were carefully explained to participants, who provided written informed consent before entering the study. These participants were taken from various drug rehabilitation centers located in Lahore-Pakistan and were using diazepam from last 6-8 months. As per the data provided by the participants in the questionnaire form, the majority of the participants were using Relax (5 mg), Valium (2 mg), Cerelium (5 mg). The participants for the current study were using above-mentioned benzodiazepines for atleast 2 months. The study was conducted in accordance with the Declaration of Helsinki and with prior approval from the Ethical Review Committee of the University of Health Sciences, Lahore. Urine, blood, hair and cerumen samples were obtained from the participants that were using diazepam and nordiazepam. Sampling for urine (5-10 mL) was done in gold-top tube (preservative free) whereas for blood (2 mL), grey top tube containing sodium flouride and potassium oxalate as preservative was used. Hair sampling (100mg) was done by cutting scalp hair from crown region. Sampling of cerumen was performed using Raucotupf cotton swabs (size-S) (LohmannRauscher, Neuwied, Germany) from both ears.. QuEChERS method was used as sample preparation technique and Ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS-MS) was used for quantitative analysis.

Results & Discussion: No analyte signals were detected in blank cerumen samples using LC–MS/MS. Validation data is shown in table # 1 below. The analyses of the spiked cerumen samples showed that reliable detection of all analytes was possible. In all cases of recent drug use of benzodiazepines, the corresponding cerumen was positive for diazepam (0.3-25 ng/mg) and nordiazepam (0.5- 50ng/mg). In five cases, where drugs could only be detected in urine, cerumen was also found to be positive. Similarly, in cases where only hair was positive cerumen still showed promising results (0.2-2.5 ng/mg for diazepam and 0.3 -5.0 ng/mg nordiazepam).

Table # 1: Validation Results (Cerumen)

Limit of detection	0.1 ng/mg
Limit of quantification	0.2 ng/mg
Precision (%CV)	2.6-8.0
Carry Over	No carry over for the current study

Conclusion: The results of the present study suggest that cerumen can be a promising alternative for drugs of abuse testing. The detection time window of cerumen is obviously in excess of that of urine but not as long as with hair. The study also concludes that using earwax as a matrix for the detection of drugs, it was possible to detect the analytes recently administered (within a week), as well as drugs administered some months before.

First evidence of protonitazene, a novel synthetic opioid, in human hair

SOHT F-P-06

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Background & Aims: The nitazene analogs are today considered as highly potent candidates for a new opioid crisis as for example, protonitazene or etonitazene are 100 and 500 times more potent than heroin. Protonitazene is a synthetic benzoimidazole opioid of the nitazenes class, developed in the 1950s as an effective analgesic, but never released in the market due to severe side effects and possible dependence. Despite its increasing use as a new psychoactive substance starting from 2019, its detection in human hair of intoxicated or deceased consumers has never been reported. We present the development and validation of a specific procedure to identify the drug in hair by LC-MS/MS.

Methods: Hair specimens were collected from 2 deceased subjects (femoral blood at 0.8 and 0.1 ng/mL, respectively and non-specific asphyxia signs at the autopsies) in jail (Mayotte Island, Indian Ocean) and from 2 other living persons who were in the same cell. After decontamination with dichloromethane, hair specimens were cut into very small segments (< 1 mm). The method involves overnight incubation at 40 °C in 1 mL pH 9.5 borate buffer of 20 mg of hair in presence of 1 ng fentanyl-d5 used as internal standard. Drugs are then extracted with 5 mL of organic solvents (diethyl ether / dichloromethane / hexane / isoamylic alcohol; 500/300/200/5, v/v). The chromatographic separation was performed using a HSS C18 column with a 15 min gradient elution. Linearity was verified from 1 to 100 pg/mg. The limit of detection was estimated at 0.1 pg/mg. Hair samples were analyzed by LC/MS-MS (Waters Xevo TQSmicro).

Results & Discussion: Protonitazene was identified at 70 and about 7600 pg/mg in the whole head hair specimens of two male subjects deceased from acute drug overdose. In the living subjects, concentrations of protonitazene were 14 and 54 pg/mg. The universal hydroxylated metabolite, 4'-OH nitazene, was not available as a standard and could therfore not be included in the method.There is no reference value for protonitazene in human hair after drug exposure and therefore these results are difficult to interpret in terms of doses used and frequency of use. Although the 14 to 70 pg/mg range is consistent with what was expected given the low dose of protonitazene used to get a pharmacological effect and the comparison with the limited data (< 200 pg/mg) available for isotonitazene, another drug from the same class, the concentrationat 7600 pg/mg appears surprizing by its elevated numeral. It can be indicative of sweat-mediated external contamination. Excessive sweating is often associated to the death agony phase in case of acute opioid poisoning.

Conclusion: To the best of the authors' knowledge, this study is the first contribution to the specific identification of protonitazene in hair using a validated methodology. However, the toxicological significance of the measured concentrations remains difficult to establish given the paucity of the literature on this topic.

Navigating challenges: Investigating New Psychoactive Substances (NPS) analysis in hair

SOHT P-07

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Background & Aims: Since the beginning of 2023, the United Nations Office on Drug and Crimes (UNODC) have reported nearly three hundred new psychoactive substances (NPS). NPS are produced by modifying the chemical structure of known drugs of abuse to increase potency and avoid laws. Due to their steady fast-growing manufacturing, forensic laboratories face numerous challenges when reporting NPS. The constant alteration of their molecular structures prevents timely production of reference materials and the development of suitable analytical methodologies, often leaving toxicologists behind schedule. A potential solution to determine trends in NPS use is by performing hair analysis due to its extended window of detection. However, the analysis of NPS in hair can be particularly complex. Low levels, non-standardized methodologies and complex interpretation are some of the issues of hair analysis, especially when coupled to NPS. The aim of this study is to provide a comprehensive review of the most recent literature encompassing the quantification of NPS in human hair and any issues encountered.

Methods: A systematic literature search of peer-review articles published since 2023 with a focus in quantification of NPS in hair analysis was conducted. Searching tools included PubMed and Scopus databases. Search terms comprised of "new psychoactive substances", "NPS", and "hair analysis". This review focused on five NPS subclasses: synthetic opioids, synthetic benzodiazepines, synthetic cathinones, synthetic cannabinoids and dissociative anesthetics. Parameters evaluated included sample preparation (sample size, segmentation, and decontamination), extraction methods and analytical instrumentation used.

Results & Discussion: A total of 24 articles spanning from 2023 to March 2024 were assessed in this review. In 22% of the articles within this research, they reported having analyzed general NPS while 37% focused on synthetic cannabinoids, 15% on dissociative anesthetics, 11% on synthetic cathinones, 11% on synthetic opioids, and 4% on synthetic benzodiazepines. Articles which focused on two subclasses were calculated individually for the overall percentages. On average, ~30mg of hair sample was used for the analysis. Decontamination was preformed using multistep washes which included the use of both aqueous and organic solvents. Extraction methods used were digestion with M3® Reagent (9%), liquid-liquid extraction (5%), solvent swelling (64%), and ultrasonication (23%). Ninety-five percent of analytical methods used liquid chromatography tandem mass spectrometry (LC-MS/MS) for quantification of NPS in hair. The analyte concentrations reported are in the range of 0.2-2000 pg/mg. Without a large amount of sample available low drug concentrations may be undetectable. The need for greater sample sizes is increased when segmental analysis is performed. Issues surrounding contamination from sweat and smoke are prevalent with the need for more readily available hair reference materials and internal standards for NPS. There is a limited knowledge on comprehensive quantitative data for NPS in hair and more large-scale epidemiological studies are needed. Additionally, multi-analyte methods, detecting more than one class of NPS are needed, however, different analytes are bound to require different extraction protocols which may limit the sensitivity of a multi-class method.

Conclusion: This study provides a review of the literature assessing the quantification of in NPS in hair and potential arising issues since the beginning of 2023 until March 2024. Overall, hair analysis can improve the community's knowledge in NPS trends and help building a relevant timeline of events considering its segmentation capabilities and long window of detection. However, it is important to acknowledge intrinsic issues when analyzing NPS in hair such as available sample size, low drug concentration, and limited large epidemiological studies.

SOHT P-08

The analysis of methylamphetamine and its metabolites in post-mortem hair samples

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Background & Aims: Methylamphetamine (MA) is a highly addictive stimulant drug prevalent in medico-legal death investigative casework in Australia. It is accepted that drugs detected in hair indicate exposure; however, the possibility of external contamination must always be considered and remains a key interpretative limitation. This is even more pertinent due to the increasing rates of smoking MA as the preferred route of administration. The Society of Hair Testing recommends decontamination procedures and the analysis of metabolites in hair to help identify drug users. However, there are no guidelines regarding the analysis of specific metabolites of MA. We have developed an analytical method using liquid chromatography-tandem mass spectrometry to detect MA, amphetamine (AMP), and para-hydroxy-methylamphetamine (p-OH-MA) in hair to assess the potential of these analytes to differentiate use and external contamination.

Methods: Hair samples from deceased persons who were positive for MA in peripheral blood (≥ 20 ng/mL) were sourced from the Victorian Institute of Forensic Medicine. Hair segments (≤ 3 cm) closest to the root end were washed with dichloromethane and water, and the washes were retained for analysis. Dry hair samples (~ 10 mg) were then subjected to a methanolic micro-pulverization for extraction, and the supernatant evaporated to dryness prior to reconstitution in mobile phase (95:5, A:B). The reconstituted hair extracts were separated on a UCT Selectra® Aqueous C18 HPLC Column (100 x 2.1 mm, 3 µm) by gradient elution of 5 mM aqueous NH4CO3 (pH 3.5) (A) and 0.1% HCOOH in methanol (B) over 11.7 min. Analyte detection was achieved using a Sciex Triple Quad 6500+ system operating in scheduled multiple reaction monitoring with positive electrospray ionization. The method was validated according to ANSI/ASB Standard 036, Standard Practices for Method Validation in Forensic Toxicology. All data processing and statistical analyses were performed using SCIEX MultiQuant® v3.0.3 and GraphPad Prism 10.0.0, respectively.

Results & Discussion: Validation was satisfactory and the lower limits of quantitation were 0.01 ng/mg for MA and AMP, and 0.001 ng/mg for p-OH-MA. Hair samples from 63 deceased persons were analyzed and the median hair concentrations of MA, AMP, and p-OH-MA were 13 ng/mg (range = 0.015–49; n = 51), 1.1 ng/mg (range = 0.018–44; n = 60), and 0.020 ng/mg (range = 0.0012–0.38, n = 62), respectively. Concentration ratios of MA and its metaboli-

tes in hair exemplified the need for sensitive analytical methods; indeed, the ratios of AMP/MA were 0.0080–0.18 (median = 0.075, n = 49) and the ratio of p-OH-MA/MA were 0.00037–0.020 (median = 0.0014, n = 50). Nonetheless, the concentrations of MA and other targeted metabolites in hair were strongly positively correlated (r = .7202 to .8641, p < .001) suggesting similar modes of incorporation. Moreover, the wash/hair ratios were indicative of external contamination; for example, the wash/hair ratios were 0.053–4.6 (median = 0.35, n = 50) for MA, 0.036–2.6 (median = 0.24, n = 56) for AMP, and 0.038– 3.7 (median = 0.21, n = 50) for p-OH-MA. Simple dichotomization of the wash appearance ('clean' versus 'soiled') further demonstrated possible post-mortem contamination with significantly higher wash/hair ratios of MA and AMP amongst 'soiled' hair samples (p = .005 and p < .001, respectively).

Conclusion: The hair concentrations of MA and its metabolites were determined in a cohort of 63 deceased persons positive for MA in blood. The oxidative metabolite of MA, p-OH-MA incorporated at very low concentrations, constitutes a potential marker of MA use like the hydroxy-cocainics. Most wash/hair ratios were high (i.e., > 0.1) suggesting incorporation from external source(s), perhaps even from post-mortem contamination. Therefore, the distribution of hair concentrations cannot be considered representative of living populations and future studies should include clinical hair samples from MA users.

Detection of xylazine in hair specimens collected from a high-risk population using LC-MS/MS analysis

SOHT P-09

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Background & Aims: Xylazine is a sedative approved for veterinary medicine but not approved for human consumption. While some actively seek out the drug, xylazine has recently been found as an adulterant, unbeknownst to the user. Negative side effects of xylazine include CNS and respiratory depression, hypotension, hypothermia, high blood glucose levels, miosis, hypotension, and necrotic skin ulcerations that can lead to amputation. According to the DEA, there were 149 reported cases involving xylazine in 2015, but that number jumped to 8,938 in 2021. Little is known about the prevalence in the population using hair testing to indicate exposure to xylazine. This study aims to report the prevalence and measured concentration of xylazine in hair from a high-risk population using liquid chromatography tandem mass spectrometry.

Methods: Hair specimens received at a national reference laboratory in the United States were surveyed. Only hair specimens that had a positive initial immunoassay screen for fentanyl were included. Twenty milligrams underwent sample preparation, including an acetone wash, powdering, acid digestion and solid-phase extraction. The presence of xylazine was determined by liquid chromatography tandem mass spectrometry in positive ionization mode. Method validation followed ASB 036 guidelines including precision, accuracy, linearity, LOD, and assay interferences.

Results & Discussion: Of the 438 presumptive fentanyl positive samples, 98 specimens had detectable xylazine levels (22.37%). Concentrations of xylazine detected ranged from 1 pg/mg to 2734 pg/mg (median=14.64 pg/mg). Other drugs detected along with xylazine included amphetamines (49%), cocaine (22%), methadone (12%), opiates (5%) and cannabinoids (23%).

Conclusion: Xylazine is a growing threat in the United States and is quickly becoming a concern in the general population. In this study, xylazine was found in a significant number of fentanyl positive hair specimens, along with co-exposure to other substances. Accurate identification of xylazine in hair can help shed light on xylazine prevalence and trends in forensic toxicology testing.

Segmental analysis of multi-illicit drugs in head hair of patients treated in a rehabilitation program

SOHT P-10

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Background & Aims: Illicit psychoactive drug abuse remains a major concern around the world due to potentially severe health consequences. Studies of illicit drug use often rely on self-report measures of use. Their potential inaccuracy can occur due to the social undesirability of illicit drug use, perceived negative consequences of disclosing use, poor recall, and the variable content of illicit drugs. The role of hair testing as an alternative or comple-

mentary matrix has expanded across the spectrum of toxicological investigations and as a result hair samples are routinely collected during drug rehabilitation programmes. In this study, it was aimed to perform segmental hair analysis for illicit drugs and their metabolites in hair samples of 98 drug users who were beginning a controlled drug rehabilitation program.

Methods: The study population (n = 98) is involved in a rehabilitation program in Alcohol and Drug Addiction Treatment Center, Ankara, Türkiye. Authentic hair samples were collected from 98 patients who were beginning a controlled drug cessation program on the 1st, 14th, 21st and 30th day after they declared that they had stopped illicit drug use. The hair samples were cut into 1, 2 or 3 cm sections (a total of 217 segments) depending on their lengths. The hair segments were prepared using a liquid-liquid extraction method and then analysed using a validated liquid chromatography tandem mass spectrometer (LC-MS/MS) method for detection of Δ9-tetrahydrocannabinol (THC), 11-nor-9-carboxy-Δ9-THC (THCCOOH), 11-hydroxy-THC (THCOH), amphetamine, methamphetamine, methylendixyamphetamine (MDA), methylendioxymethamphetamine (MDMA), morphine, codeine, 6-monoacetylmorphine (6-MAM), cocaine, benzoylecgonine, ecgonine methylester, buprenorphine, pregabalin, gabapentin and new psychoactive substances (13 synthetic cathinones and 94 synthetic cannabinoids).

Results & Discussion: The mean age of the patients was calculated as 28±5 years. 94 (95.9 %) were male and 4 (4.1 %) were female. When the education levels were investigated, 17.3 % of the patients had primary education, 46.9 % had secondary education, 30.6 % had high school, 4.1 % had associate degree and 1% had bachelor's degree. At least one illicit drug was found positive in all cases. 81.6% (n=177) of the hair segments were positive for methamphetamine, 62.7% (n=136) for amphetamine, 69.6% (n=151) for morphine, 65.0% (n=141) for codeine, 69. 1% (n=150) for 6-MAM, 17.1% (n=37) MDMA, 6.0% MDA (n=13), 10.1% (n=22) for cocaine, 10.6% (n=23) for BZG, 1.8% (n=4) for EME, 27.2% buprenorphine, 22.1% (n=59) pregabalin and 1.4% gabapentin (n=3). THC and its metabolites THCOOH and THC-OH were not in all samples. The positivity in hair segments of the cases varied from distal to proximal. It is remarkable that cannabis, the most widely used illicit substance in the world and in Türkiye, was not detected in this sample. When the percentages of use are analysed, it can be concluded that the use of illicit substances trends to opiate and methamphetamine in this study.

Conclusion: It is the first hair study conducted in rehabilitation patients in Türkiye. The results showed that the combination of illicit drugs and their metabolites, and segmental hair analysis in illicit drug users can provide a retrospective impression of drug exposure and use patterns.

SOHT P–11

Hair analysis as powerful tool for evaluation of drug abuse in psychiatric population. Focus on the cathinone MDPHP in a case of intoxication with severe agitation.

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Background & Aims: Toxicological hair analysis is a remarkable diagnostic instrument for the assessment of past drugs use/abuse use both of therapeutic and abused drugs. Besides the well-known application to many aspects of forensic toxicology, it can be a useful tool for the diagnosis of past drugs use for various purposes, including clinical diagnosis of drugs abuse, for example in patients with psychiatric disorders. Due to the huge number of drugs present on the illicit market, effective analytical methods, able to detect a wide range of substances in hair samples, possibly with a single extraction and analytical run, are the most desirable. In the past years, we developed a liquid chromatography-High Resolution Mass Spectrometry LC-HRMS screening method for the wide-range detection of xenobiotics in hair matrix. It was applied to hair samples from psychiatric patients to evaluate their toxicological behaviour, adhesion to the therapeutic protocol, concordance with self-report of drugs used, and the possible use of new psychoactive substances (NPS). Since the early 2000s, NPS pertaining to different chemical classes have appeared in the European recreational drugs market, including unregulated ring-substituted cathinone derivatives. In the recent few years, the cathinone 3,4-methylenedioxy-alpha-pyrrolidinohexanophenone (MDPHP) was frequently identified in confiscations and in biological samples from intoxication, even lethal in Italy. Twelve MDPHP intoxications with agitation symptoms, assessed by urine and blood analyses, were reported in Lombardy region in the last year by the Italian Early Warning System. The aim of our study was hence to retrospectively evaluate the intake of drugs and NPS in psychiatric patients by hair analysis. In particular, we describe the case of a subject showing a severe dissociative state, that reported the use of MDPV during a chem-sex party. He was submitted to toxicological analysis of hair to identify the drugs used that could have been the trigger of his agitation and dissociation state.

Methods: Hair samples were cut from the posterior vertex, as close as possible to the scalp. Where possible, hair samples were divided into 1.5-centimeter sections, to better assess the subject's drug intake history. Sample pretreatment procedure involved a first decontamination step, followed by the digestion of the keratin matrix in two steps, for the extraction of various classes of drugs of abuse, including natural and synthetic cannabinoids, cathinones and other classes of NPS. The extract was directly injected in liquid chromatography coupled with high-resolution mass spectrometry (LC–HRMS) using a benchtop Orbitrap instrument.

Results & Discussion: More than seventy hair samples belonging to psychiatric patients (70% men, age from 18 to 55) were analysed. The presence of several therapeutic drugs and drugs of abuse, both declared and undeclared from the subjects, was assessed in the hair samples. Analytes were identified by exact mass, correspondence of isotopic cluster and retention times, using a constantly updated home-made built database, containing more than 200 compounds. More than fifty subjects were found positive for cocaine and metabolites; about 10% of subjects were positive for opiates, 15% for THC; several benzodiazepines and antipsychotic drugs, both prescribed and non-prescribed, were detected in 15% of hair sample. Ketamine and MDMA were also identified in 4% of cases. No significant differences were found between different hair sections belonging to the same subject. In two cases methylmethcathinone (MMC) was identified. In particular we report the case of a 33 year old male patient, suffering from a mood disorder, narcisistic personality disorder, and a history of alcohol and substance abuse, in treatment for HIV. In January 2024 he took part in a party using alcohol and different drugs. Suddenly, he presented a severe dissociative state and he was hospitalized in a psychiatric unit for approximately 50 days. A sample of hair was collected 3 weeks after admission. The hair strand was 10 cm long, and was divided into 7 sections. The analysis of all the sections of hair showed the presence of cocaine (>5 ng/mg in all the sections), cocaethylene (0.32-0.65 ng/mg) and of the synthetic cathinone MDPHP (0.12-0.65 ng/mg). As in the majority of other MDPHP intoxication cases, the subject wrongly reported the use of methylendioxypyrovalerone MDPV, not determined in the hair.

Conclusion: The analysis of hair, carried out by a high-throughput technique as LC-HRMS, proved once again to be a powerful tool for the assessment of drugs intake. The constantly updated database, alongside with the possibility of a retrospective analysis of previously acquired data files, provide an essential tool for toxicological investigation in hair. The study demonstrated the high prevalence of drugs of abuse among psychiatric patients. Moreover, hair analysis is useful for the correct diagnosis of the substances taken, often unknown also to the users, and for a better understanding of the possible concause of the disorder. Furthermore, by carrying out toxicological analyses on selected patients, it is possible to better study the effects of those substances whose effects are not yet fully known.

Trends in mephedrone detection and reporting

SOHT P-12

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Background & Aims: The use of mephedrone in the UK appears to have declined over recent years. We therefore undertook a data analysis to determine if mephedrone is still a detectable substance in hair, and whether the levels seen by the laboratory have changed over the past few years. Data was also reviewed to see what drugs are commonly seen together with mephedrone.

Methods: Data from all samples analysed in 2021, 2022 and 2023 was reviewed to ascertain how many samples tested positive for mephedrone during these periods. The data from the positive samples was further reviewed to determine if / what other substances were also detected above the cut off.

Results & Discussion: Of the cases analysed by the laboratory between the beginning of 2021 and the end of 2023, 10 tested positive for mephedrone. Many other drugs, including amphetamine, cannabis, cocaine, MDMA and opiates, were also detected in these cases; the most prevalent of which was cocaine, with cocaine being detected in 90% of these cases.

Conclusion: Mephedrone can be detected in hair and is still a drug that is being used recreationally, together with other illicit substances, and is most likely to be used together with cocaine.

Analyzing hair surface to detect the use of pepper spray

SOHT P-13

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Background & Aims: In the case of a homicide, a potential pepper spray attack should be investigated. In addressing this question, examining hair samples for the presence of the active ingredient capsaicin can offer a promising avenue. The structure of head hair should be well suited for the deposition of aerosols and droplets on its surface.

However, for a conclusive interpretation of the analysis results, it should be considered whether a positive finding for capsaicin could potentially be attributed to other causes, such as the ingestion of capsaicin-containing foods. Data from control subjects are necessary for this purpose.

Methods: Strands of hair were washed with acetonitrile. The rinse solution was analyzed using tandem mass spectrometry. Data acquisition was conducted using a Sciex QTRAP 5500 LC-MS/MS system (ESI+, MRM mode) paired with a Shimadzu HPLC equipped with two solvent delivery units (LC-20AD XR), a communication bus module (CBM-20A), an autosampler (SIL-20AC XR), a degasser (DGU-20A 5R), and a column oven (CTO-10AC). The Analyst 1.6 software was employed for this purpose. Capsaicin and dihydrocapsaicin were determined using deuterated capsaicin as an internal standard. The developed analytical method underwent validation following the protocols outlined by the German Society of Toxicological and Forensic Chemistry.

Results & Discussion: In the real case, capsaicin and dihydrocapsaicin were detected on the hair samples of the victim, indicating a spray attack. Initial results from comparative studies involving voluntary subjects showed that capsaicin and dihydrocapsaicin were not found, or were found only in low concentrations, in hair analyses without exposure to pepper sprays.

Conclusion: The presented method allows for the analysis of capsaicin and dihydrocapsaicin as components of pepper sprays. Hair samples, in our assessment, are suitable for detecting a pepper spray attack. The procedure is a preliminary approach to evaluate pepper spray exposure through analysis of the hair surface. To conclusively prove spray application forensically and obtain data on the influence of capsaicin-containing foods, further data are currently being collected as part of a broader study. In addition to analyzing the rinse solution, an analysis of the target substances will be performed after extracting embedded portions from the hair matrix, as is common in the typical procedure for examining drugs in hair.The presented method allows for the analysis of capsaicin and dihydrocapsaicin as components of pepper sprays. Hair samples, in our assessment, are suitable for detecting a pepper spray attack. The procedure is a preliminary approach to evaluate pepper spray exposure through analysis of the hair surface. To conclusively prove spray application forensically and obtain data on the influence of capsaicin-containing foods, further data are currently being collected as part of a broader study. In addition to analyzing the rinse solution, an analysis of the target substances will be performed after extracting embedded portions from the hair matrix, as is common in the typical procedure for examining drugs in hair.

SOHT P–14

A comparative evaluation of Wondfo® multi-drug hair test cup with Neogen® elisa in human hair: Comparison with gas chromatography or liquid chromatography-mass spectrometry

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Background & Aims: This study compared Wondfo® Multi-Drug Test Cup with Neogen® ELISA response for the initial screening of drugs of abuse in previously tested donor specimens. Previously tested donor specimens were re-evaluated with Gas Chromatography Mass Spectrometry (GC/MS) or Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) to determine quantitative values of drugs in each specimen. The following drugs were tested utilizing both screening methods with their corresponding cutoff values: Amphetamine (500 pg/mg), Methamphetamine (500 pg/mg), MDMA (500 pg/mg), Oxycodone (200 pg/mg), Fentanyl (40-Wondfo®, 100 pg/mg), Cocaine (500 pg/mg), Opioids (200 pg/mg). All donor specimens were archived specimens that previously tested positive at Omega Laboratories Inc. Our aim is to evaluate the instant test cup when compared to a laboratory-based screening test method currently utilized by Omega Laboratories Inc.

Methods: Wondfo® Multi-Drug Test Cup: 30 mg of previously tested donor hair were analyzed utilizing Wondfo® Multi-Drug Test Cup.

Principle of Wondfo® Mul-Drug Test Cup: This test employs competitive immunochromatography method to detect the presence of specified drugs in human hair specimens.

During the test, the hair specimen mixes with the drug-specific monoclonal antibody conjugate and flows across the membrane. When sample drug levels are zero or below the target cutoff, drug monoclonal antibody conjugate binds to the respective drug-protein conjugate immobilized in the Test Region (T). This produces a colored band in the Test Region (T) that, regardless of its intensity, indicates the negative result.

When drug concentrations in the sample are at or above the target cutoff, the drug in the sample binds to the respective drug monoclonal antibody conjugate and prevent the respective drug monoclonal antibody conjugate from binding to the respective drug-protein conjugate immobilized in the Test Region (T). This prevents the development of a colored band in the Test Region (T), regardless of its intensity, and indicates the preliminary positive result.

Neogen® Enzyme Linked Immunosorbent Assay (ELISA): 10-15 mg of previously tested donor specimens were pulverized then heated with acidified methanol for 1 hour. Specimens were then tested using Neogen® Enzyme Linked Immunosorbent Assay (ELISA) which is a competitive microplate immunoassay for the determination of drugs of abuse listed above. The appropriate calibrators and controls were run alongside all donor specimens.

Confirmatory test utilizing GC/MS or LC/MS/MS: 20 mg of previously tested donor specimens were pulverized. The appropriate amount of internal standard was added to all specimens and calibrators/controls. Solid Phase Extraction (SPE) was performed on all donor specimens. The donor specimens were then injected onto GC/MS or LC/MS/MS with appropriate calibrators and controls to determine the quantitative value of drug present in each specimen.

Results & Discussion: Percent correct on both methodologies

	n=	Wondfo® (% correct)	Neogen® ELISA
(+) Amphetamine	29	96.5%	93.1%
(+) Methamphetamine	30	93.3%	93.3%
(+/-) MDMA	29	93.1%	86.2%
Oxycodone	28	89.2%	92.8%
Fentanyl	27	100%	96.2%
Cocaine	30	93.3%	93.3%
Morphine	19	89.4%	100%
Codeine	17	82.3%	76.4%
6-AM	27	96.2%	96.2%
mean % correct		92.5%	91.9%

Evaluation of donor specimens compared to confirmatory results:

One ELISA false negative outside +/- 25 % of cutoff level for oxycodone. One Wondfo® and ELISA false positive outside +/- 25 % of cutoff for cocaine. All other false negative/positives are within +/- 25% of cutoff levels.

Conclusion: Both the Wondfo® Test Cup and Neogen ELISA yielded similar initial test response when compared to confirmatory quantitative values. Like most screening methods, being 100% accurate around the cutoff can be a challenge. Most false negative/positives were within +/- 25% of cutoff levels. The Wondfo® instant cup performance was comparable with the Neogen® ELISA laboratory test. Currently performing more testing on previously tested donor specimens utilizing both methodologies so more statistical analysis can be performed on a larger sample size.

Development of a simultaneous detection and quantification method of three HIF stabilizers (molidustat, roxadustat and vadadustat) in multiple keratinized matrices: First detection of molidustat in an authentic doping case

SOHT P-15

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Background & Aims: According to the World Anti-Doping Agency (WADA) rules, following an adverse analytical finding (AAF) in urine, it is up to the athlete to prove his innocence.

In this study, the authors report the case of a professional athlete with an AAF in urine for molidustat. Molidustat or BAY-3994 is a hypoxia-inducible factor prolyl hydroxylase inhibitor (HIF-PHI) and is being tested in clinical trials as a new treatment for anemia associated with chronic renal failure. This molecule is designed to mimic hypoxia and increase endogenous EPO production. In this context, this substance is banned at all-times in sport by the WADA and classified as S2. Peptide hormones, growth factors, related substances and mimetics.

The athlete challenged an anti-doping rule violation involving molidustat. On the advice of his lawyer and after discussion with the toxicologist, head hair (4 cm, black), beard hair and fingernail clippings, sampled 4 weeks after the urine test, were sent to the laboratory.

Methods: The identification of molidustat in hair has never been described in the literature. In response to future requests concerning HIF-PHI, a method for identifying and quantifying three molecules from this class (molidustat,

roxadustat and vadadustat) in hair was developed. 30 mg of hair or nails were incubated (15h, 40°C) in a buffer at pH 8.4 in the presence of 1 ng of testosterone-D3, used as an internal standard. After addition of an ethyl acetate/diethyl ether mixture (80/20), the organic phase was evaporated, recovered in 30 µL of initial phase and then 5 µL was injected into the LC-ESI-MS/MS system. Separation was performed on a BEH C18 column (100 mm × 2.1 mm, 1.7 µm).

The transitions chosen to quantify the molidustat were m/z 315>207 (quantification) and 315>137 (confirmation).

Results & Discussion: Linearity was tested for molidustat, roxadustat and vadadustat in hair from 1 to 1000 pg/mg, with a limit of quantification of 5 pg/mg, 1 pg/mg and 10 pg/mg, respectively. For the three analytes, intra-day and inter-day precision results have a CV lower than 20%, which satisfies laboratory's standards. According to the presented doping case, head hair analysis revealed the presence of molidustat at 135 pg/mg. This sample covers the period of the urine test. Molidustat was positive in beard hair and fingernails at 55 and 40 pg/mg, respectively. Due to its novelty and because of the lack of data in literature, molidustat concentrations cannot be easily interpreted. It is not possible to correlate these findings with hypothetical ingested quantities because of the lack of information in literature.

Conclusion: The authors presented a method for the simultaneous identification and quantification of three HIF-PHI in hair, with the first identification of molidustat in an authentic doping case. The lack of data in the literature makes it difficult to interpret the results in hair, but this could indicate repeated exposure, without being able to give either the dose or the frequency of consumption.

SOHT P-16

Enhancing analytical techniques for the measurement of pyrethroids in hair

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Background & Aims: Human exposure to toxic contaminants occurs through various sources in their daily activities and the use of consumer products. These exposures can be assessed directly via biological monitoring, and although blood and urine are mainly used as biological matrices, hair is an innovative tool to do so but its use is limited due to lack of data and the unknown limits of this matrix. The present work assesses atmospheric pressure chemical ionization (GC-APCI) versus electronic impact ionization (GC-EI) for the determination of pyrethroid insecticides in hair for environmental analysis.

withdrawn

Methods: 24 hair samples were collected and stored at -20°C. They were grinded and pyrethroids of interest solvent-extracted. The organic extracts were then analyzed for 12 parent pyrethroids (allethrin, bifenthrin, cyfluthrin, cypermethrin, deltamethrin, imiprothrin, lambda-cyhalothrin, permethrin, phenothrin, prallethrin, tetramethrin, transfluthrin) by GC-APCI-MS/MS and GC-EI-MS/MS, and the results compared.

Results & Discussion: Analysis using GC-APCI-MS/MS demonstrates higher sensitivity, enabling the division of LOD and LOQ levels by at least tenfold compared to GC-EI-MS/MS (LOQEI=10 µg/L and LOQAPCI=0.02 µg/L for phenothrin and LOQEI=0.05 µg/L and LOQAPCI=0.005 µg/L for transfluthrin). Pyrethroids were detected in hair through GC-APCI-MS/MS at concentration levels below previous LOQ values obtained with the GC-EI-MS/MS approach. The augmented sensitivity boosts detection rates and quantification frequencies, while the enhanced specificity improves peak resolution, thereby facilitating peak integration. Additionally, it reduces the required sample mass, consequently reducing matrix effect.

Conclusion: The GC-APCI-MS/MS method is very promising as it enables the detection of pyrethroids in hair samples at much lower concentration levels, hence improving the interpretation of contaminant levels in this matrix.

SOHT P-17

Investigation of interferences in negative hair samples on determining concentrations of EtG in hair

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Background & Aims: A sensitive LC-MS/MS method for analysis of ethyl glucuronide (EtG) in hair has been developed and fully validated. The four-point calibration curve showed linearity over the range of concentrations from 5-2000 pg/mg hair. Intra- and inter-day precision and accuracy were lower than 10 and 15%, respectively. However, the interfering peaks in blank samples presented a challenge for determining EtG concentrations close to cut-off value where interferences constitute 10-40% of the lowest calibration level. The validated method has been applied to analyse various authentic hair samples from twelve different abstinent grown-up individuals. The aim of the study was to shed the light on the interferences issue in hair at low concentrations.

Methods: Twelve different hair samples from abstinent grown-ups have been collected either by routine sample collection kit or cut at the hairdresser's. Two sets of samples were analysed, where one set as blank and the other one as spiked to the lowest calibrator at 5 pg/mg. The samples were weighed, cut into segments and washed either with isopropanol (non-treated hair, n=9) or with isopropanol followed by phosphate buffer (dyed or striped hair, n=3). Aliquots of 30 mg (3 cm) washed and powdered hair were added with 1.5 ml of water with or without EtG and 50 µL of internal standard (EtG-d5). The samples were incubated in a water bath at 37 °C for 18 hours. Supernatant was cleaned up and concentrated by SPE OASIS MAX cartridges (60 mg, 3 mL). The extract was evaporated to dryness in a vacuum centrifuge at 45 °C and the resulting dry extract was reconstituted with 60 µL of ultra-purified water. Analysis was performed with an UHPLC-MS/MS system composed of an ACQUITY I-Class chromatograph coupled with Xevo TQ-XS tandem quadrupole mass spectrometer (Waters, USA) in negative electrospray mode. The chromatographic separation was performed with an ACQUITY UPLC® HSS T3 (1.8 mm, 2.1 × 100 mm) using 0.1% of formic acid in water and methanol as mobile phases at a flow rate of 0.6 mL/min. The column temperature was set at 50 °C. Injection volume was 5 µL.

Results & Discussion: Interferences were present in all blank samples and constituted 10-40% of the lowest level of calibration (5 pg/mg), where most of them were between 17 and 22%. Those interfering peaks present a challenge for determining EtG concentrations close to cut-off value and present a risk for both underestimation and overestimation of the concentrations.

In case of higher interference in the blank sample than in the patient hair sample being analysed, the result would be an underestimation of the real EtG concentration in the hair sample. Equal interferences in both blank and patient hair sample would not result in any overestimation or underestimation. If the interference in the hair sample were to be higher than that in the blank hair (i.e. higher than 10%, since this is the lowest interference observed), the result would be an overestimation of the real EtG concentration in the sample.

One way to identify EtG in a sample and define false positives is to assess the transition ion ratio as a ratio of area of the quantifier- and qualifier-ion. The validation of our method has shown that the maximum deviation for the transition ion ratio of the calibration curve was 30%. In blank samples, the ratio of the interferences area varies between 40 and 100% from quantifying reference. The target ion ratio tolerance for our method is therefore set to 30%.

Conclusion: The fully validated method for EtG in hair has been used for analysis of various authentic hair samples from abstinent individuals in order to determine how often interfering peaks occur. The results have shown that the interferences occur in three out of twelve analysed samples and constitute 10-40% of the lowest level of calibration. These interferences would contribute to the underestimation of EtG concentrations or in some cases overestimation if the patient hair sample contains interferences higher than 20% relative to the lowest level of calibration.

In order to avoid issues with determining EtG concentrations close to the cut-off value, one should be careful with choosing the type of hair used for the spiking of the calibrators and controls. This supposedly blank hair should be tested first. On the other hand, one cannot be sure if the patient sample contains interferences from the hair itself or the patient has actually consumed certain amount of alcohol. Therefore it is of great importance that transition ion ratio is assessed for samples with concentrations close to the cut-off value. Another question yet remains to be discussed and resolved, and that is if one should use grown-up or children's hair for calibrators and controls spiking.

Microextraction by packed sorbent clean-up for the determination of selected synthetic cathinones in hair samples

SOHT P-18

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Background & Aims: The use of synthetic cathinones (SC), also known as "bath salts," has been increasing in recent years, leading to a growing concern about their effects on public health. Different clean-up approaches have already been studied by different authors. However, microextraction by packed sorbent (MEPS), a miniaturized form of solid-phase extraction, has not been explored extensively for the determination of SC in hair samples. Therefore, we propose the optimization of the MEPS procedure as sample clean-up method followed by gas chromatography mass spectrometry for the determination of these analytes in hair samples.

Methods: Hair samples were washed consecutively with dichloromethane, deionized water and methanol to remove dirt and externally deposited material. After drying, hair was scissor-cut into small fragments. Fifty milligrams of hair were incubated overnight in the presence of 1 M NaOH at 45 °C, after which the extracts were neutralized and subjected to MEPS using a C8-SCX cartridge. The compounds were analyzed underivatized by GC-MS in the SIM mode. MEPS conditions were conditioning (250 µL of methanol followed by 250 µL of water), sample loading (24 x 700 µL of hair extract) and elution (7 x 100 µL of 2% NH4OH in acetonitrile).

Results & Discussion: The procedure resulted in acceptable recoveries (15-59% for all analytes), and allowed reaching lower limits of quantification (LLOQs) of 100 pg/mg for 4-MDMB, 4-MNEB and 4- MDMP and of 200 pg/mg for the remaining analytes (4-CEC, 4-MNEP, 4-MDEB, 4-MDMH, ethylone, alpha-PVP, penthylone, 4-Cl-PVP, MPHP and MDPV).

Conclusion: In this work the optimization of the MEPS technique coupled to GC-MS for the determination of selected synthetic cathinones in hair samples is presented. The method was considered suitable for the analysis of these compounds in hair, providing acceptable recoveries and obtaining low limits of quantification.

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SOHT P-19

Drug facilitated sexual assault: modus operandi elucidated by hair analysis

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Background & Aims: In a context of drug facilitated sexual assault (DFSA), a photographer invited women to his studio for a photo shoot and made them drink large amounts of alcoholic beverages. Eleven people lodged complaints. The events would have spanned over several years.

The hair of 7 victims have been analysed for the most frequently used substances in DFSA (benzodiazepines, antidepressants, sedatives and neuroleptics). These investigations showed how crucial hair analysis can be, even several months or years after the event.

Methods: Hair samples were collected by a policeman. The delay between the event and the sampling ranged from 10,5 months to of 24 months. Considering the significant time periods, hair segmentation was adapted for each sample.

Hair strands were twice decontaminated in methyl chloride, segmented and incubated in Sorensen buffer with clonazepam-D4. After overnight incubation at 56°C, liquid-liquid extraction and filtration on PTFE 0.2 µm were performed. An aliquot was evaporated to dryness and the dry extract was dissolved in the mobile phase. Samples were analysed by HPLC Surveyor ThermoFisher - MS/MS TSQ Quantum ThermoFisher. A large panel of benzodiazepines, antidepressants, sedatives and neuroleptics commonly used in a DFSA context were researched.

Results & Discussion: In 4 out of 7 cases, extremely low concentrations of alprazolam were detected on the segment corresponding to the events. No other substances were detected.

Victims n°1, 2 and 3 had hair sampled with respectively 10,5 months, 13 months and 17-to-20-months delay. Alprazolam was the only substance detected on the segment contemporary to the event, respectively at 12 pg/mg, 5 pg/mg and 2 pg/mg. These concentrations may correspond to a single ingestion of alprazolam.

For victim n°4, alprazolam was detected on the two segments potentially contemporary to the events (<1 pg/mg and <1 pg/mg), as well as on the earlier segment, 2-month older than the event (1 pg/mg). Not all the hair is synchronised because of growth-phase differences; consequently, over time, the substances become increasingly dispersed between nearby segments.

No substances were detected in the hair samples of victims n°5, 6, and 7. Considering the delay (respectively 20, 24 and 24 months) and the fact that victims 5 and 6 had coloured hair, a possible single intake of alprazolam may no longer be detectable.

Conclusion: This case illustrates once again the importance of hair analysis in DFSA. These analyses revealed the presence of the same substance on the segment corresponding to the events for 4 victims, at concentrations consistent with a single intake. This suggests a repeated modus operandi.

Reference concentrations and distribution of 7-aminoclonazepam in hair following a single dose of clonazepam

SOHT P-20

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Background & Aims: The benzodiazepine clonazepam is frequently implicated in drug-facilitated sexual assault (DFSA). In DFSA cases where the substance is no longer detectable in blood and urine, hair analysis is an important tool to reveal exposure to clonazepam. To distinguish single from repeated doses, established reference concentrations of clonazepam and its main metabolite 7-aminoclonazepam in hair following a controlled single dose are essential. This study aimed to establish reference values of clonazepam and 7-aminoclonazepam in hair following a single dose of clonazepam and to investigate the drug distribution in hair collected at four different sampling time points.

Methods: The study protocol was approved by the Reginal Ethics Committee in Linköping, Sweden (number 2010/41-31) and all participants gave their informed consent to participate. Eighteen volunteers (15 women) consumed a single dose of either 0.5 (n=8) or 2 mg clonazepam (n=10). Head hair samples were collected from each participant 14, 30, 60, and 120 days after administration. The hair samples were aligned, cut into 1 cm segments (10 mg), and washed with isopropanol and two times with water. Each hair segment was pulverized and incubated in an extraction medium (methanol, acetonitrile, 2 mM ammonium formate, 25:29:46, pH 5.3) overnight at 37°C. After filtration, the hair extracts were analysed by ultra-high performance liquid chromatography–tandem mass spectrometry using a validated limit of quantification of 1.0 and 0.50 pg/mg for clonazepam and 7-aminoclonazepam, respectively.

Results & Discussion: All analysed hair samples were positive for 7-aminoclonazepam, while clonazepam could not be detected in any of the hair samples. Assuming an average hair growth rate of 1 cm/month, the highest concentrations of 7-aminoclonazepam were found in the expected segment in most of the hair samples with lower concentrations in the adjacent segments.

The time-resolved drug distribution profiles across the four sampling time points showed that the highest concentrations were detected in the hair samples collected after 30 and 60 days. The lower concentrations in hair samples collected after 14 days can be explained by the fact that there is still some positive hair below the surface of the scalp and that hair was cut 1-2 mm above the scalp. In hair samples collected after 120 days, the drug was more dispersed in several segments due to irregular hair growth. In the hair samples collected after 30 and 60 days, the concentrations of 7-aminoclonazepam ranged from 1.1 to 10 pg/mg (median: 2.1 pg/mg) in the low-dosing group (0.5 mg), while the concentrations in the high-dosing group (2 mg) were 3-4 times higher ranging from 2.2 to 35 pg/mg (median: 9.6 pg/mg).

The cumulative concentrations for each sampling time were lowest at the first sampling time and consistent across the three later sampling time points, indicating that 7-aminoclonazepam is not subject to significant sweat contamination or washout over time.

Conclusion: Reference values were established for 7-aminoclonazepam in hair from 18 participants who ingested a dose of either 0.5 or 2 mg clonazepam, while clonazepam was not detected. This shows that 7-aminoclonazepam is a good marker of clonazepam exposure and that it is crucial to include this metabolite in the analysis to confirm a single exposure to clonazepam. A significant difference between the two administered doses of clonazepam and the incorporated amount of the metabolite 7-aminoclonazepam was observed. The time-resolved drug distribution profiles showed that analysis of 1 cm segments can assess the time of a single exposure of clonazepam and that the hair samples should be collected 1-2 months after the exposure to provide the best conditions for detection and interpretation of the hair results.

Quantitative 31P NMR spectroscopy investigation in hair of bisphosphonates as banned substance in sports

SOHT P-21

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Background & Aims: Bisphosphonates (BPs) are a class of pharmaceutical drugs approved by FDA for use in human health as well as veterinary medicine. There are many books and review articles available that describe the chemistry, pharmacology, and applications of BPs in clinical and veterinary medical use. [, ,]

Bisphosphonates are prohibited drugs according to the International Federation of Horseracing Authorities (IFHA). [Article 6 of the International Agreement on Breeding, Racing and Wagering]. Any administration of a bisphosphonate to a racehorse less than 4 years old is an unacceptable practice because of an high risk of serious injury or death from deleterious effects on bone growth and strength as a consequence of such use.

These compounds are used for the treatment of navicular syndrome and related diseases in horses and are divided in two groups: non-nitrogen-containing bisphosphonate drugs (e.g., tiludronic acid) and nitrogen-containing bisphosphonate drugs (e.g., zoledronic acid). In forensic toxicology, hair is used as a research material to detect various substances, particularly in detecting drugs for a long period of time post administration. []: Due to their very polar and strong chelating characteristics, determination of bisphosphonates represents a real analytical challenge for routine screening.

According to a recent study about successful use of hair samples for detection of BPs in horses, a method of liquid chromatography-tandem mass spectrometry was used.[]

Methods: Quantitative 31P NMR Spectroscopy is a valid method in analytical chemistry for detection of phosphorus containing compounds, but still not used for detection of BPs in hair as a method of choice.

31P-NMR spectroscopy is widely used to study biological structures under native conditions, which is useful for studying hair.

Preliminarily , we used an in silico computational study of the toxicity of BPs by Yasara software to use a molecular simulation method.

Results & Discussion: Phosphorus-31 NMR, which has been widely applied since the early days of in vivo NMR. The 31P isotope (I=1/2) is 100% naturally abundant with a sensitivity of 6.63% that of 1H. Computer simulation model was used to mimic in vivo 31P NMR spectroscopy. Alongside with 31P magnetic resonance imaging both are important applications of this nucleus. 31P signals from inorganic phosphate, can be observed in whole-cell preparations, intact tissues, and whole bodies and can provide information of the cells. Both empirical and theoretical correlations between measured coupling constants, 31P chemical shifts, and structural parameters have provided an important probe of the conformation and dynamics of various phosphorus-containing compounds, particularly BPs.

Conclusion: We report the results of an in silico investigation of the binding of a series of bisphosphonate drugs to hair keratin using 31P nuclear magnetic resonance spectroscopy. The 31P NMR simulation shows molecular dynamics quantitative results of binding interaction with hair keratie that bisphosphonate groups bind to protein.

SOHT P-22

Markers for phenyltetrahydroimidazothiazole (levamisole/tetramisole) ingestion in cocaine-positive hair samples

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Background & Aims: Cocaine, the most commonly used illicit stimulant in Europe, is predominantly adulterated with phenyltetrahydroimidazothiazole (PTHIT). PTHIT, which was marketed as tetramisole ((S)/(R)-PTHIT) for anthelmintic purposes, is the racemic form consisting of levamisole ((S)-PTHIT) and dexamisole ((R)-PTHIT). Frequent use of PTHIT along with cocaine has been reported to carry a risk of clinical complications such as agranulocytosis, neutropenia, thrombocytopenia and vasculitis. The use of these substances can be retrospectively monitored by hair analysis. However, the main challenge in the interpretation of hair analysis results is to differentiate between ingestion as opposed to external contamination of hair. One approach is the distinction on the basis of metabolite-to-parent-drug ratios (MR). For this purpose, first, the prevalence of (S)-PTHIT (levamisole) versus (S)/(R)-PTHIT (tetramisole) and their metabolites in seized cocaine samples adulterated with PTHIT should be investigated. Second, the presence of PTHIT metabolites in hair samples positive for cocaine and PTHIT was investigated. For both investigations, analytical methods using chiral and non-chiral liquid chromatography-tandem mass spectrometry (LC-MS/MS) were developed and fully validated. The main aim of this study was to elucidate whether MR could be considered as markers for ingestion of cocaine laced with PTHIT in contrast to external contamination.

Methods: Two hundred cocaine samples adulterated with PTHIT seized in the Canton of Zurich between 2013 and 2018 were firstly analyzed for the presence of (S)/(R)-PTHIT enantiomers using a validated chiral LC-MS/MS method. Secondly, the samples were analyzed for the PTHIT metabolites aminorex, 4-hydroxy-PTHIT (4-OH-PTHIT), 2-hydroxy-PTHIT (2-OH-PTHIT), 4-phenyl-2-imidazolidinone, and 2-oxo-3-(2-mercaptoethyl)-5-phenylimidazolidine by non-chiral LC-MS/MS. Two cohorts of cocaine- and PTHIT-positive hair samples, previously interpreted as "indicative of predominant cocaine use" (n=54) and "indicative of predominant cocaine contamination" (n=33), were analyzed by non-chiral LC-MS/MS for the above-mentioned metabolites and MR in the two cohorts were compared.

Results & Discussion: The chiral LC-MS/MS method for the analysis of PTHIT enantiomers was successfully validated. Chiral analysis of the seized PTHIT-laced cocaine samples revealed that 89% actually contained racemic (S)/(R)-PTHIT, 10% of the samples contained a mixture of (S)-PTHIT and (S)/(R)-PTHIT and only 1% of the samples

contained levamisole (S)-PTHIT. PTHIT contents ranged from below the lower limit of quantification (LLOQ) to 82 % (m/m; mean: 11.8 %). All seized cocaine samples were negative for all tested PTHIT metabolites. The LC-MS/MS method for the analysis of PTHIT metabolites in hair samples was successfully validated for all metabolites except for 2-oxo-3-(2-mercaptoethyl)-5-phenylimidazolidine. PTHIT concentrations in hair samples ranged from below LLOQ to approximately 13,000 pg/mg; they were statistically not different between the two groups "cocaine use" versus "cocaine contamination". The analysis of PTHIT metabolites in the hair samples revealed that aminorex was negative in all samples. 4-OH-PTHIT, 2-OH-PTHIT, and 4-phenyl-2-imidazolidinone were detectable in 94 %, 84 %, and 53 % of hair samples at concentrations ranging from below LLOQ to 112.28 pg/mg, below LLOQ to 32.45 pg/mg, and below LLOQ to 85.66 pg/mg, respectively; they were detected in samples with PTHIT concentrations of ≥30, ≥60, and ≥360 pg/mg, respectively. In hair samples of the cohort "cocaine use", PTHIT and metabolite concentrations displayed a significant linear correlation. MR for 4-OH-PTHIT/PTHIT in the cohort "cocaine use" and "cocaine contamination" ranged from 0.00037 to 0.055 (mean: 0.011) and 0.000059 to 0.0036 (mean: 0.0008), respectively. MR for 4-OH-PTHIT/PTHIT and 2-OH-PTHIT/PTHIT, but not for 4-phenyl-2-imidazolidinone/PTHIT, were statistically significantly higher in the cohort "cocaine use" compered to "cocaine contamination" with p values of <0.0001, 0.0033, and 0.9391, respectively.

Conclusion: The study demonstrates that (S)/(R)-PTHIT (tetramisole) but not levamisole appears to be the predominant adulterant for cocaine between 2013 and 2018 in the Canton of Zurich. 4-OH-PTHIT, 2-OH-PTHIT, and 4-phenyl-2-imidazolidinone, but not aminorex, have been identified as major PTHIT metabolites in hair samples. Concentration ratios of PTHIT metabolite to PTHIT have been evaluated in hair samples with the two interpretation cohorts "cocaine use" versus "cocaine contamination". Due to the highest abundance and strongest statistical difference in the two cohorts, the 4-OH-PTHIT/PTHIT ratio seems to be the most promising marker to indicate ingestion of cocaine adulterated with PTHIT. The implementation of this marker in interpretation criteria for cocaine and PTHIT positive hair samples could be helpful for the distinction between cocaine and PTHIT ingestion versus external contamination.

Intimate through hair analysis!

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SOHT P-23

Background & Aims: If contamination issues in children, due to their thinner and more porous head hair fiber, have been documented since 2010 (1,2), there is quite no literature regarding cross contamination between intimate adults. In a context of child abuse, hair specimen collected on both parents were analyzed for drugs of abuse and pharmaceuticals. Parents were treated with different antidepressant drugs, but the antidepressant treatment of each subject was also observed through the hair analyses of his/her partner. We report a case where cross contamination hypothesis between intimate adults was of first evidence, even if incorporation routes cannot be specified.

Methods: Two hair locks were collected on both parents. Father's hair was blond in color and 4cm in length. Mother's hair was brown in color and more than 20cm in length. Samples were segmented after decontamination (twice for two minutes, using methylene chloride) and cut into small segments (< 1mm). Targeted drugs were extracted from powdered hair using direct methanolic solubilization, in presence of deuterated internal standards. Hair and washing baths were analyzed according to our HPLC-MSMS methods, the first being dedicated to cannabinoids only and the second one to other drugs of abuse and pharmaceuticals.

Results & Discussion:

Concentration (pg/mg)	0-2cm	2-4cm	Washes
THC-COOH	7.1	7.2	Not detected
Sertraline	> 200	> 200	10
Mirtazapine	6	11	Not detected

Table 1: Drugs detected in the father's hair

Concentration (pg/mg)	0-3cm	3-6cm	Washes
THC-COOH	2.5	2.9	Not detected
Mirtazapine	> 200	> 200	11
Sertraline	< 2	3	Not detected

Table 2: Drugs detected in the mother's hair

Both parents were identified as active cannabis users with the detection of carboxylated metabolite of THC in the tested hair sections. All other common drugs of abuse remained undetectable.

Regarding our targeted pharmaceutical screen results (see Tables 1 and 2), sertraline and mirtazapine were detected in the hair collected from both parents. When compared to previously published paper (3), sertraline concentrations (see Table 1) suggested the repeated use of this pharmaceutical by the father within the time period covered by the hair tests and does not contradict his sertraline prescription. For the mother, mirtazapine results (see Table 2) suggested, when compared to previously published paper (3), the repeated use of this antidepressant, within the time period covered by the hair section tests and does not contradict her mirtazapine prescription. In both cases, the corresponding low sertraline and mirtazapine concentrations measured in the washes did not suggest external contamination as the major route of incorporation into their hair specimen.

On another hand, trace amounts of sertraline were detected in the mother's hair, when low mirtazapine concentrations were detected in the father's hair. Sertraline and mirtazapine concentrations were considered as low when compared to people treated with these two antidepressant drugs (3) and suggested the occasional exposure of the father to mirtazapine and the occasional exposure of the mother to sertraline. Although the occasional use of mirtazapine by the father and the occasional use of sertraline by the mother cannot be totally excluded, results interpretation in this particular situation led us to consider more consistently the hypothesis of cross-contamination between two intimate adults.

These residual amounts of sertraline and mirtazapine in both cases were detected with increasing concentrations from the root to the tip ends, similar to the typical distribution profiles observed in the hair of children passively exposed through contaminated environment or close contacts with drug users, the oldest hair sections being more contaminated than the newest ones. In children, it is well known and described that head hair is more sensitive to external contamination because hair fiber is thinner and more porous than those of adults. Even if hair of adults would be less sensitive to this phenomenon, this hypothesis cannot be excluded in these reported cases.

Conclusion: Our case illustrates probable cross-contamination between two intimate adults where the antidepressant treatment of each subject was also observed through the hair analyses of his/her partner.

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SOHT P-24

Optimized sample preparation for low level determination of the alcohol marker EtG from hair using UPLC-MS/MS analysis

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Background & Aims: Hair analysis is growing in popularity due to the non-invasive nature of sample collection and can provide advantages over other more widely used matrices, such as assessment of prolonged exposure and potential timelines. However, as a solid matrix sample preparation can often be lengthy and labour intensive. Minor ethanol metabolites such as ethyl glucuronide (EtG) and ethyl palmitate (EtPa) can be measured in hair as direct markers of alcohol consumption. As suggested by the Society of Hair Testing (SoHT) in 2019, EtG determination from hair is the preferred marker for the assessment of abstinence. This poster aims to demonstrate improved workflow and clean-up of hair matrix to allow low level EtG determination required for alcohol abstinence testing.

Methods: Hair samples (10 mg) were subjected to micro-pulverized extraction using the Biotage® Lysera bead mill homogenizer. Drug release was evaluated using both dry and in-solution pulverisation options. Once in liquid form, sample extracts were further cleaned-up using polymer-based solid phase extraction (SPE) in 96-well plate format. Both strong and weak mixed-mode anion exchange SPE chemistries were compared for the extraction. Subsequent analysis was performed using a Waters ACQUITY Premier UPLC coupled to a Xevo TQ Absolute triple quadrupole mass spectrometer. Negative ions were acquired using electrospray ionization operated in the MRM mode. Chromatographic separation compared both HILIC and reversed phase for optimum performance and sensitivity.

Results & Discussion: Initial method development focussed on investigation of LC mobile phases and column chemistry to achieve good chromatographic performance while providing adequate negative ion sensitivity. An HSS T3 column provided good reversed-phase chromatography albeit with acidic mobile phase composition, resulting in slightly reduced negative ion sensitivity. HILIC chromatography yielded good sensitivity generally at the expense of compromised chromatographic peak shape. The biggest impact on this assay was down to the early elution of interferences and lack of resolution afforded by HILIC chromatography. Further method optimisation was provided using reversed phase LC.

Evaporative effects were investigated using a range of traditional SPE elution solvents. The results demonstrated between 15-25% analyte signal reduction depending on exact solvent combination.

Matrix homogenization was investigated after standard washing protocols. Both dry and in solvent hair pulverisation was evaluated. 10 mg of hair was extracted with up to 1 mL of H2O to effect efficient analyte solubilisation of target analyte from the matrix.

Sample extraction and clean-up was performed using polymer-based mixed-mode strong and weak anion exchange SPE chemistries targeting the carboxylic acid moiety on the analyte. Wash and elution optimization resulted in analyte recoveries greater than 80% for both chemistries. Suppression profiles were reduced resulting in matrix factors greater than 0.7, depending on exact protocol. Final workflow streamlining evaluated SPE bed reduction, minimum elution volumes and direct injection compared to evaporation/reconstitution protocols. Signal reduction from the lack of concentration afforded by evaporation was offset by analyte losses on evaporation and bed mass reduction to 10 or 2mg fixed well plates. Both approaches presented different advantages with respect to simplicity and time vs absolute sensitivity but ultimately achieved the required LOQs. Calibration curves were constructed using hair spiked between 1-100 pg/mg to cover the range of chronic excessive consumption and abstinence. Results demonstrated good linearity and coefficients of determination (r2) values greater than 0.99. LoQs were determined to be below the required SoHT guidelines for abstinence cases of equal to or below 5 pg/mg of hair.

Conclusion: This poster presents simplified approaches for the low-level detection of EtG in hair matrix, as required in abstinence cases. Scalability of SPE from 30 mg > 2 mg formats demonstrated high reproducible recoveries and low matrix factors. The strong anion exchange (AX) chemistry, along with optimized elution solvents provide an excellent extraction option allowing the elimination of evaporation steps for improved workflow when using 10 mg or 2 mg Mikro SPE formats.

Distribution of metformin in the hair of long term users

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Background & Aims: Drugs and toxins that enter the body can bind to hair. Metformin, as a hypoglycemic drug, has a wide range of users and occasional cases of overdose leading to death. The aim of this study is to use hair samples to summarize the hair content and distribution patterns of long-term metformin users.

Methods: Hair samples of 5 metformin users (1.0~1.5g per day for over 2 years) were collected. The hair sample was segmented along the hair axis using a razor blade, ground into powder, and soaked in methanol to extract metformin. The hair extracts were analyzed by LC-MS/MS at MRM mode.

Results & Discussion: Three hair samples (H1, H2 and H4, 4~8 cm in length) were cut into 5~10 mm segments and quantitative analyzed. Metformin was positive for all the segments, with the content ranging from 0.12 to 1.37ng/g. In dyed hair (H2), metformin decreased significantly from hair root to tip (from 0.80ng/mg to 0.19 ng/mg). Metformin content in the three beard samples (M1~M3) was 0.52~2.63 ng/mg. Dose dependence was observed that metformin content in beard decreased by 61% during the week of withdrawal. The black hair and white hair from the same users were detected separately, indicating that the content of metformin in black hair was significantly higher than that in white hair. For H1,the average content was 0.69 ng/mg in black hair and 0.09ng/mg in white hair. H5 was 0.70 ng/mg in black hair and 0.16 ng/mg for white hair.

Conclusion: Metformin was positive in the hair and beard samples of all 5 participants in the experiment. Dyeing may cause damage to the hair matrix and loss of metformin.On the same user, dose dependence was observed. A significant difference of metformin content was found in the black and white hair from the same person, supporting that melanin is an important binding site in the process of metformin entering hair.

Poster gallery - CT F-P-1 to P-26

10:00 - 10:30 Wednesday, 4th September, 2024

CT F-P-01

Two-year report of pediatric unintentional cannabinoid intoxication in a Sicilian Pediatric Hospital

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Background & Aims: The impact of cannabinoids, alone or in combination with other drugs, in the case of unintentional pediatric intoxications, is still poorly documented. We reported the admissions for suspected cannabis intoxication in children under 10 in a 2-year period (11/2021-09/2023) in a Pediatric Hospital in Palermo, Sicily.

Methods: All children aged <10 years admitted to a pediatric emergency department for cannabis intoxication were included in this study. The symptoms were evaluated and a first-level toxicological screening on biological fluids was performed. A second level of toxicological confirmation was executed when required and metabolites monitoring in biological fluids has been performed as support to the clinical detoxification treatment.

Results & Discussion: Twenty-one children were admitted to the pediatric emergency department in the observed period for suspected cannabis intoxication, although in very few cases parents declared the possibility of drug ingestion, delaying the diagnosis. The average age of the children was 1.5 years old, with twenty children (95%) between 11 months and 2 years old, and one child around 9 years old. Among the cases, the judicial authority took charge of sixteen of them (76%). Eighteen children (86%) were positive for cannabinoids. Of them, two were also positive for ethanol (11%), one was also positive for cocaine and a benzodiazepine (6%), and one for a barbiturate (6%). Three (14%) were negative for cannabinoids but positive for methadone, cocaine in combination with benzodiazepine, and cocaine in combination with amphetamine. 50% of children who presented a positivity in the first level screening in blood, presented a positivity also in urine. All the forensic cases were confirmed in a second-level analysis.

Around 50% of the patients presented tachycardia, while most of the were responsive only to painful stimulus, and all of them presented various symptoms including inconsolable crying, irritability, acute cerebral edema, syncope, vomiting, soporous state, fever, poor reactivity, and many others. Psychomotor agitation and aggression were observed when the cannabis was combined with cocaine and other drugs. 50% of the children presented various types of blood count alterations.

No correlation between plasma concentrations and symptoms could be found. However, 50% of the cases positive for cannabinoids, presented high hematic concentration of the main secondary cannabinoid metabolite, 11-nor-9-carboxy-THC, indicating that the drug assumption preceded the hospitalization by at least a few hours.

Conclusion: Unintentional cannabis ingestion by children is a serious public health concern. In addition, sometimes its ingestion is combined with other substances. The results showed that the diagnostic suspicion should be always combined with a drug screening for the correct identification of the specific substance. Furthermore, the second level of analysis is crucial for treatment monitoring and identify unexpected drugs. This study shows that clinicians should consider cannabis intoxication in any child with sudden onset of lethargy or ataxia, especially in cases of ambiguous familiar or social context. Drug screening tests could prevent unnecessary, invasive, and expensive procedures.

CT P-02

Deadly delay: Paracetamol overdose case in a resource-constrained setting

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Background & Aims: Paracetamol is an over-the-counter medicine widely used as a painkiller, commonly found in the medicine cabinets of almost every household. Its widespread availability makes it the most common drug involved in accidental and deliberate poisoning cases. Overdosing on Paracetamol can have severe consequences. However, its antidote, N-acetylcysteine (NAC), is highly effective when used properly. Nevertheless, it shows less efficacy in cases of delayed presentation. We report a fatal case of deliberate self-poisoning with Paracetamol after delayed presentation for treatment.

Methods: It is a case report of a young woman who presented to the emergency department after a suicide attempt by paracetamol overdose. The quantification of paracetamol in the patient plasma was performed by spectrophotometric method. We used the Rumack-Matthew nomogram to assess the potential risk of hepatotoxicity.

Results & Discussion: A 22-year-old female medical student, weighing 59 kg, was hospitalized for a massive ingestion of paracetamol approximately 15 hours prior to her admission. The patient consumed 24 tablets, each containing 500 mg (total = 12 grams). About 6 hours after a massive single ingestion, she had developed vomiting and abdominal pain. Her medical history revealed a background of depression, self-harm, and previous suicide attempts. The quantification of paracetamol in the patient plasma revealed a level of 25 mg/l, 18 hours after the ingestion (the risk of serious liver toxicity is significant at the threshold of 17.68 mg/L). The oral N-Acetyl-Cysteine was started after her admission. However, the patient vomited the first doses given as she was experiencing emesis. As the oral NAC was the only formulation available, the treating physician had to wait until the parents could obtain the IV formulation from outside the country to use it. Meanwhile, symptomatic management was carried out. But the mental and physical state of the patient continued to deteriorate. About 36 hours after paracetamol ingestion, the transaminases reached 3000 IU, and the prothrombin activity percentage dropped down to 10%. The patient received transfusions of Fresh Frozen Plasma (FFP) to treat the coagulation disorders. On the third day, the patient's mental state deteriorated due to brain oedema, and she was intubated. On day 6, the patient received NAC in a continuous IV infusion. However, she died within 8 hours after the start of the IV therapy.

According to the American Association for the Study of Liver Diseases (AASLD) guidelines, treatment with IV and oral NAC effectively prevents hepatotoxicity, regardless of the initial serum acetaminophen level, if it is started within 8 hours of ingestion.

Acoording to Yarema and al, the IV formulation is preferred for patients presenting within 12 hours of ingestion, as it provides a higher blood level of NAC compared to oral administration. However, the total dose of IV NAC administeration (300 mg/kg over 20 hours) is lower than that of oral administration (1,330 mg/kg over 72 hours), and delivered a lower amount to the liver. The IV formulation also carries an increased risk of anaphylactic reactions.

In this case, the NAC administered 15 hours after the alleged ingestion was inefficient as the patient was experiencing vomiting and mental deterioration. It's worth noting that the NAC administered was a dietary supplement as the medication was not available.

Conclusion: In conclusion, this case highlights the critical importance of timely intervention in cases of paracetamol overdose. The unfortunate delay in obtaining the intravenous formulation of NAC, compounded by the unavailability of the medication and subsequent reliance on a dietary supplement, ultimately proved fatal for the patient. This tragic outcome underscores the urgent need for improved access to essential medications and healthcare resources to prevent avoidable deaths from overdose-related complications.

Key role of patient interview and toxicology analyses in revealing carbamazepine overdose

CT P–03

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Background & Aims: Carbamazepine (CBZ), is a major first line antiepileptic drug (AED) in the treatment of various kinds of epilepsies and is also approved as a mood-stabilizer for bipolar disorder and the treatment of trigeminal neuralgia. Because it has been on the market for decades, knowledge about its profile makes it the most prescribed drug for the focal epilepsy in many countries, and is widely used across Europe, Africa, South America, and Asia, where it represents a good compromise between cost, availability, and effectiveness. Indeed, CBZ is listed on the WHO model list of essential medicines. Approximately 1014 tons are annually consumed worldwide, making it one of the highest detected pharmaceutical residues in aquatic environments.

Carbamazepine shows good results in the treatment of bipolar disorder. However, its high toxicity makes it a second option when there is no response to lithium. The first overdose to carbamazepine was reported in 1967, and according to the US Poison Control Center, there are nearly 2000 cases each in the US. Furthermore, literature reports cases of carbamazepine misuses linked to its euphoric effects.

Carbamazepine poisoning can result in severe neurological and cardiovascular complications. An overall mortality rate of 13% was documented and reported in a cohort study of 427 patients with CBZ intoxication, with an average CBZ intake in lethal cases of 23.6 g. This study aims to evaluate the critical roles of clinical interviews and toxicological analyses in identifying and managing carbamazepine intoxicaton, with a specific focus on a clinical case.

Methods: This study reports a case of a patient admitted to the emergency department for alteration in mental status. The patient was misdiagnosed, and since it wasn't initially considered an overdose, samples weren't collected immediately. It was the psychiatric team's involvement that prompted a drug screening test for further assessment. Consequently, blood and urine samples weren't collected until six hours after admission for toxicological analyses. The toxicology department conducted a thorough medical interview with the patient and his family member and performed analyses using the immunoassay method (EMIT) on the provided samples.

Results & Discussion: The case involves a 25-year-old patient who presented to the Emergency Department for Altered consciousness. Initially perceived as a drug addict, the patient received intravenous hydration and was discharged and referred for psychiatric follow-up after less then 7 hours of hospitalization. Subsequently, he was oriented to the Toxicology department for a substance abuse screening. The patient was experiencing nystagmus, ataxy and confusion. The interview with a his sister revealed that the patient was being treated for schizophrenia with antipsychotic medication, and there had been a recent change in the medication regimen the day before admission. The family member provided us the two last prescriptions and was also carrying the last prescribed medication of the patient.

We reviewed the two prescriptions to check for potential interactions between the medications, using the Practical Guide to Medication (Dorosz, edition 2020). Although both of the prescriptions included Carbamazepine, there was a change in dosage from 200 mg once daily to a controlled-release form (400 mg) administered three times daily.

Analyses using EMIT technique were performed on urines and blood simple both collected 6 hours after the patient admission to the Emergency room. While urine drug screening returned negative results, serum carbamazepine level was elevated at 27 mg/L (exceeding the toxicity threshold). Consequently, the patient was referred back to the emergency department with the indication of multiple-dose activated charcoal therapy and cardiac function monitoring.

The enterohepatic cycle of the molecule and its depressive effect on intestinal motility contribute to its prolonged stay in the digestive tract. Thus, multiple-dose activated charcoal therapy is indicated in such cases. The patient later admitted that he had ingested a blister pack of tablets in search of stimulant effects. The patient may have confused Carbamazepine with trihexyphenidyl, a medication used in his regimen to counteract the antiparkinsonian syndrome related to phenothiazine drug he was taking. Trihexyphenidyl is often misused by psychiatric patients for its stimulant properties. The patient was asked and he confessed overdosing on the stimulant medication with a yellow packaging and showed the Tegretol packaging. As the patient had limited education and as both parkidyl (trihexyphenidyl 2 mg) and Tegretol (Carbamazepine 400 mg XR) had the same packaging color, we assumed that he may confused between the two of them.

Conclusion: It is imperative for healthcare professionals to conduct thorough clinical interviews and toxicological analyses to effectively diagnose and treat intoxication cases. Furthermore, the case emphasizing the need for collaboration between Emergency and toxicology departments. By adopting a patient-centered and multidisciplinary approach, healthcare providers can effectively manage medication intoxications and reduce the stigma surrounding mental health conditions in emergency settings.

CT P-04

Outbreak of methanol poisoning linked to clandestine alcohol production

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Background & Aims: Historically, incidents of mass methanol intoxications have been associated with alcohol prohibition, stemming from the proliferation of clandestine alcohol production. Incidents of methanol poisoning persist due to the consumption of cheap or counterfeit alcoholic beverages, particularly in regions with limited access to legal alcohol or low-income countries. This case report aims to present an outbreak of methanol poisoning that occurred in Tlemcen by the end of the year 2023.

Methods: This is a case report of patients hospitalized for a decline in their general condition after consuming an alcoholic preparation. Methanol analysis was performed on blood samples using GC-FID.

Results & Discussion: In late December 2023, 14 men presented to a local hospital with symptoms including nausea, vomiting, headache, abdominal pain, and vision impairment. Interviews with the patients revealed that they all consumed an alcoholic preparation the day before. The patients' conditions deteriorated rapidly, with 9 of them succumbing to the poisoning and 5 others being evacuated to Tlemcen University Hospital. The latter patients were aged between 33 and 74 years old; two patients had a medical history of hypertension and AIDS. They exhibited a deterioration in consciousness, bilateral unreactive mydriasis and high blood pressure, 3 patients experienced

mild dyspnea, and one other showed focal signs of paraparesis and aphasia. No abnormalities were found on brain CT scans, while laboratory analyses revealed metabolic acidosis with high anion gap, high lactate levels, hypokalemia, dehydration, low platelet and prothrombin levels. As the antidotes fomepizole and therapeutic ethanol were unavailable in the country, therapy consisted of symptomatic treatment, dialysis, platelet concentrate infusion and oxygen therapy for the patient experiencing desaturation in oxygen blood pressure. Two patients died on the same day, and a third one after 5 days of hospitalization. Two patients survived the intoxication but suffered optic nerve damage resulting in irreversible bilateral blindness. Blood samples of two patients, taken at the admission, were sent to the Toxicology laboratory. After protein precipitation by addition of trichloroacetic acid 10% (v/v) and centrifugation, the supernatant was injected to a Gas Chromatography system coupled to a Flame-Ionization Detector. Qualitative analysis confirmed the presence of methanol (the retention time for methanol = 1.8 minutes, LOD = 12 mg/L).

Conclusion: Unconsciousness and metabolic acidosis in methanol intoxication cases are associated to a high risk of mortality. Immediate initiation of medical treatment and hemodialysis is essential, articulately in presence of mental state alterations, visual disturbances, metabolic acidosis, or a history of methanol ingestion. Furthermore, the lack of access to specific antidotes such as fomepizole and therapeutic ethanol can delay or impede effective treatment, potentially resulting in poorer outcomes for affected individuals.

Intoxication of 3 adolescents by oral nicotine pouches

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CT P-05

Background & Aims: Adolescents' use of nicotine pouches, as alternative to smoking and dipping, has increased since 2020. As a result, the frequency of reports of nicotine intoxication has increased drastically as well. Oral nicotine pouches contain variable quantities of nicotine. Consequently, various symptoms of acute nicotine poisoning could appear rapidly, and may include nausea and vomiting, abdominal pain and cramping, headache, dizziness and confusion, rapid heart rate and high blood pressure, weakness and tremors, seizures, respiratory failure, and coma. The detection of nicotine is significant in clinical cases of teenagers who have used oral nicotine pouches.

Herein, three intoxication cases concerning adolescents (12-14 years old) who consumed oral nicotine pouches are presented. All cases were hospitalized at the local hospitals of the city of Ioannina, Epirus Greece, in 2023.

Methods: Biological samples were received (whole blood, urine) at admission to the hospital and subjected to toxicological analysis including the quantification of nicotine levels. Ethanol analysis was performed in whole blood by HS-GC-FID and a screening for drugs of abuse was performed by immunoassays, both with negative results. Afterwards, screening for the presence of common drugs and pharmaceuticals in blood and urine was performed by an UHLC-MS/MS method which includes more than a hundred analytes. The results were also negative.

Eventually, a method for the determination of nicotine in whole blood was developed on an LC-MS/MS system. More specifically, experiments were carried out by a Dionex HPLC system coupled to a Q-trap 5500+, equipped with electrospray ionization (ESI) Turbo V Source, operated in multiple reaction monitoring (MRM), and in positive mode. A reversed-phase column (C18) was used, and a gradient of ACN over H2O with a total analysis time of 7.5 minutes was applied.

Sample preparation was performed as follows: quantity of 200 µL of blood, was transferred into a 2 mL Eppendorf tube, with the subsequent addition of 200 µL of carbonate buffer (1 M, pH 9.5), and 1.0 mL of MTBE. The sample was vigorously stirred and centrifuged (10 min, 10,000 rpm). The organic layer was taken-up and evaporated to dryness under a gentle stream of N2 (40°C). The dry residue was reconstituted with 50 µL of H2O : ACN (88:12) and injected into the LC-MS/MS system for analysis.

Results & Discussion: The applied UHLC-MS/MS method was sensitive for nicotine with limit of detection (LOD) and limit of quantification (LOQ), 0.13 and 0.39 ng/mL respectively. It was applied to the analysis of the above-mentioned clinical cases of nicotine intoxication. Nicotine blood levels found 66, 134 and 266 ng/mL for these 3 cases. In two cases, urine samples were also available, and urine nicotine concentrations were 83 and 2153 ng/mL, respectively. The detected nicotine blood concentrations were all lying within the range of toxic levels concerning adolescents.

UHPLC-MS/MS analysis of blood using the developed method herein offers the potential for selective and unambiguous detection and quantitative determination of nicotine. The method comprised an efficient and quick extraction protocol using LLE and was applied in small sample volumes.

Conclusion: Nicotine pouch poisoning in teenagers can appear with adverse symptoms. Healthcare practitioners should be aware about the clinical characteristics and treatment of nicotine poisoning and include it as a possible cause when evaluating intoxicated adolescents with unclear symptoms.

CT P–06

A systematic review on alcohol abuse disorders fatality, from alcohol binge to alcoholic cardiomyopathy

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Background & Aims: Alcohol is a major contributor to worldwide disease and a leading cause of preventable death, with 3 million deaths per year attributable to alcohol. Both the occasional consumption of alcohol and its chronic intake are associated with an increased risk of road accidents, workplace productivity losses, increased medical and mental health costs, and greater rates of crime and violence. Global alcohol abuse and high rates of consumption cause multiple health problems; alcohol is considered responsible for 5.3% of all deaths worldwide. Despite the cited above negative health effects related to alcohol consumption, several observational studies have shown a significant reduction in all-cause cardiovascular mortality in subjects with low levels of alcohol consumption compared to abstainers. A recent meta-analysis with more than 16,000 patients confirmed that daily alcohol consumption of up to 25 g/d was associated with a statistically significant reduction in the incidence of cardiovascular mortality.

Methods: This systematic literature review was conducted to identify articles regarding the potentially lethal effects of alcohol consumption on heart. The methodology undertaken was based on the PRISMA statement guidelines. The literature search was carried out in October-November 2023 in electronic database as "PubMed" and "Scopus"; the following Medical Subject Headings (MeSH) were used: "cardiomyopathy alcoholic", "sudden death", "cardiac arrhythmias", "stroke", "hypertension", "Takotsubo cardiomyopathy". Boolean operators such as "AND" and "OR" were used to combine search terms. The literature search also included websites and citation searching, focusing on our research topics.

Results & Discussion: A scientific literature careful reviewed by two independent reviewers identified several pathophysiological conditions related to alcohol consumption. Fiftytwo articles is reviewed. This could make an important contribution to the scientific community not only for the medico-legal understanding of the causal mechanisms of alcohol-related deaths (in add to the gross and microscopic findings and evidences at autopsy) but also in the view of a therapeutic perspective of alcohol-addicted patients.

Alcoholic cardiomyopathy is defined as a pathological condition with structural, anatomical, and functional changes of the heart that develops in subjects who have consumed approximately 80-90 g of alcohol per day for at least a period of 5 years.

Conclusion: This systematic review highlights the pathophysiological alterations related to alcohol consumption in different ways, both in chronic alcohol consumers and in the case of alcohol binges. In this regard, population and gender studies can add further, significant kwoledge, in order to provide a fundamental support not only in clinical medicine (to undertake pharmacological therapies for alcohol-consuming patients with unknown cardiac alterations), but also in forensic medicine diagnostic of causes of death. Especially in case of cardiac sudden death, it is crucial to discriminate whether the histological findings can be attributable to a dilated cardiomyopathy or an alcoholic cardiomyopathy, which often histologically have similar characteristics.

In add, these studies outcomes could be an essential tool to highlight the correlation between alcohol and arrhythmias, which can be fatal. Further prospective studies are necessary to improve knowledge of risk factors to the development of structural and molecular modifications of vascular endothelium and cardiac structure

CT P–07

Carbon dioxide poisoning among health professionals in a surgery department

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Background & Aims: Medical and paramedical staff in operating rooms (OR) are exposed daily to various inhaled toxins, which can lead to long-term intoxication. Additionally, accidents may happened, resulting in poisoning of varying severity depending on the incriminated product and its atmospheric concentration. An incident occurred

in the Surgery Department at Tlemcen Hospital, where a CO2 leak was suspected. Carbon dioxide is commonly used in ORs for laparoscopic surgery to create space and improve visibility. This work aims to highlight the risk of toxicity related to CO2 exposure in ORs.

Methods: This is a case report detailing 38 health professionals, with a sex ratio of 0.27, who were exposed to a CO2 gas leak in the Surgery Department at Tlemcen University Hospital in November 2022. Blood samples and data were collected from these workers, and CO2 analysis was conducted using HS-GC/MS with the scan method. Initially, a pool of blood samples from both healthy smokers and non-smokers was analyzed to establish the spectrum and the normal level range of CO2 in the blood, determined by the area under the curve. Subsequently, blood samples from the exposed workers were analyzed using the same method, and the results were compared to the established normal range of concentrations.

Results & Discussion: A total of 38 professionals reported experiencing symptoms including headache (100%), sleep disturbance (47%), dyspnea (43%), and attention disorder (33%). Six females were hospitalized for more serious issues, including tachycardia, severe dyspnea, and oxygen desaturation requiring oxygen therapy. Among the 38 workers, 83% were regular visitors to the OR, spending an average of 4 hours per day. A gas leak was discovered in the CO2 piping within the operating room. However, due to the lack of air sample collection devices for analysis, confirming the exposure was not possible. Blood samples were collected, 5 days after the first incident, from the 38 professionals for toxicology analysis. It revealed elevated CO2 levels in 7 workers, including the 6 hospitalized women, while 79% had normal CO2 levels. Gasometry revealed metabolic alkalosis in all workers, even those with mild symptoms.

Conclusion: All the hospitalized patients recovered well and were able to resume normal work. Furthermore, the risk of exposure to various products in operating rooms cannot be overlooked, emphasizing the need for comprehensive surveillance of workers' safety. It is noteworthy that in the absence of air analysis, blood analysis played a crucial role in confirming the intoxication.

Optimization of high-dose methotrexate treatment in oncology: Importance of plasma concentration monitoring and toxicity management at the toxicology laboratory of CHU Sétif-Algeria

CT P–08

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Background & Aims: The dosage of methotrexate plays a crucial role in preventing its toxicity. High doses of methotrexate, particularly used in oncology, can lead to nephrotoxicity, exacerbated in case of overdose. To prevent this complication, it is essential to assess renal function before each treatment and adjust the dosage accordingly, especially in patients with impaired renal function or elderly individuals. Additionally, consideration should be given to concomitant nephrotoxic medications, either discontinuing them if possible or intensifying monitoring of renal function and methotrexate levels if their concurrent use is essential. During and after treatment, regular monitoring of urinary pH, diuresis, renal function, and methotrexate levels is crucial, along with sequential administration of folinic acid to mitigate renal toxicity. In cases of delayed methotrexate elimination with renal dysfunction, specific measures such as early folinic acid administration, alkaline hydration, or even extrarenal clearance may be necessary to prevent severe complications. This work presents the experience of the Toxicology Laboratory at CHU Sétif-Algeria, contributing to the management of patients treated with high-dose methotrexate by determining plasma concentrations of high-dose methotrexate used in onco-hematology.

Methods: Cancer patients undergoing high-dose methotrexate (HD-MTX), defined as methotrexate doses exceeding 500 mg/m2 administered intravenously, were monitored at the Toxicology Laboratory during their chemotherapy treatment. The immunoenzymatic method EMIT was employed to determine MTX plasma concentrations. MTXemia measurements were taken at 24, 48, and 72 hours post-infusion, facilitating the improved individual adaptation of folinic acid doses.

Results & Discussion: All patients in our study exhibited MTXemia levels exceeding the cytotoxicity threshold (> 5µmol/l) at 24 hours post-infusion. Subsequently, all patients underwent a second assessment at 48 hours post-perfusion. The results of this subsequent round of testing revealed that 30% of patients had values surpassing the cytotoxicity threshold (> 1µmol/l).

This monitoring allowed for the detection of patients exhibiting delayed elimination with MTXemia levels at 24 hours exceeding the cytotoxicity threshold (> 5µmol/l). Consequently, they were able to benefit from individualized adaptation of folinic acid doses based on MTX plasma concentrations, thereby enhancing treatment safety in these cancer patients by minimizing the risks of toxicity, particularly renal toxicity.

Regular monitoring of MTX-HD plasma concentrations is crucial for adjusting folinic acid doses and preventing nephrotoxic complications. It enables proactive risk management, including the identification of patients at high risk of renal toxicity and providing them with tailored follow-up.

Conclusion: Monitoring of MTX-HD plasma concentrations provides a personalized approach to optimize treatment safety in oncology patients. This practice enables proactive management of renal toxicity, ensuring improved quality of care and reduced risks associated with high-dose methotrexate use in oncology

CT P-09

Exploring new experience: Algeria's first methadone program for opioid substitution therapy

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Background & Aims: Opioid dependence constitues a significant public health issue, marked by the marginalization and stigma experienced by drug users. According to the 2023 UNODC report, opioids remain the deadliest drugs, accounting for two-thirds of deaths directly attributable to drugs, primarily due to overdose. In Algeria, the statistical report for the first half of 2023 from the National Office for Drug Control and Addiction Prevention revealed that the number of drug users receiving care in intermediate care for addiction and detoxification centers during the first quarter of 2023 reached 8,658, with 20% being women and 74% aged between 16 and 35 years. Consequently, a national substitution treatment program using methadone was established in 2021 to combat addiction. This study aims to explore the profile of opioid-dependent patients in an intermediate care center for addiction in Cheraga, Algiers, Algeria, as well as to assess the effectiveness of methadone treatment.

Methods: An observational study was conducted between March 2023 and February 2024, involving the initial 102 methadone-treated patients at the intermediate care center for addiction in Cheraga. Demographic data, opioid consumption status, and serology for hepatitis B, C, and HIV were collected.The samples were collected prior to the daily administration for patients who had been undergoing treatment for at least one month. Methadone dosage was performed using a validated, simple, and rapid method following the EMA validation protocol on GC/MS.

Results & Discussion: Among the included patients, the majority were unmarried men, with an average age of 35 years, and 81% were heroin users. Seventy-five percent of patients were seropositive for hepatitis C virus. The administered methadone dose ranged from 10 to 70 mg/day, with 60 mg/day being the most common dose (42%). Twenty percent of patients had methadone concentrations exceeding 700 µg/L, while 30% were in the upper range of the therapeutic interval (500-700 µg/L), and 50% were within the recommended therapeutic range.These values have significantly aided clinicians in adjusting doses to avoid potential therapeutic inefficacy or overdose.

The results underscore the magnitude of the opioid dependence problem in Algeria, as well as the effectiveness of methadone treatment by the evaluation of opioid abstinence and absence of withdrawal syndrome on one hand, and urine drug test to check the consumption of other psychoactive substances, and patients' quality of life on the other hand. These findings align with global data published in this context. However, challenges persist, particularly concerning dose adjustments to maintain methadone concentrations within the optimal therapeutic range, and genetic studies are recommended because there is significant interindividual variation in the genes that regulate the transport, metabolism, and binding of methadone to opioid receptors.

Conclusion: This study highlights the importance of addressing the needs of drug users in Algeria and the effectiveness of methadone treatment in this context. Further efforts are required to enhance access to treatment services and interdisciplinary collaboration to ensure the success of this program

CT P-10

A non-lethal multi-subject intoxication case due to carbofuran exposure. Detection of carbofuran phase I and phase II metabolites in blood, urine, and gastric content samples by LC-HRAM-Orbitrap-MS

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Background & Aims: Carbofuran is one of the most dangerous and toxic carbamate insecticides which, by inhibiting acetylcholinesterase, causes central and peripheral accumulation of acetylcholine, provoking a cholinergic syndrome which can lead to death by severe bronchoconstriction and/or respiratory paralysis.

Here we describe the detection of the carbofuran and its phase I and phase II metabolites in four subject biosamples by liquid chromatography - high-resolution accurate-mass Orbitrap mass spectrometry (LC-HRAM-Orbitrap-MS).

Three male and one female subjects were found on the beach one morning, comatose and hypothermic. Some witnesses stated that they had accepted a drink offered to them, and one of them had only taken a sip. All the subjects were taken to hospital. Three were admitted to intensive care and two, one male and one female, were intubated. The fourth was admitted to the medical ward. Whole blood, urine, and gastric content samples were collected from the two intubated patients (1M, 2F) on day one, and whole blood and urine from the other two patients (3M, 4M) on day three. All samples were sent to the laboratory of clinical and forensic toxicology.

Methods: An immunoassay toxicological screening for common drugs of abuse was performed on urine samples using an Instrumentation Laboratory Indiko Plus system.

Whole blood and urine samples were subjected to headspace gas chromatography – mass spectrometry (HS-GC-MS) analysis for ethyl alcohol and other volatiles, using a Thermo Scientific Triplus 500 HS - GC Trace 1300 - ISQ 7000 system.

Whole blood, urine, and gastric content samples were subjected to protein precipitation with cold acetonitrile:methanol 2:1 (v/v), after addition of an internal standard mixture, for LC-HRAM-Orbitrap-MS analysis using a Thermo Scientific Ultimate 3000 UHPLC system, equipped with an Accucore Phenyl Hexyl reversed-phase analytical column, coupled to a Thermo Scientific "Q-Exactive Focus" Orbitrap mass spectrometer instrument. MS acquisition was performed in full scan (m/z 70-1000) positive-ion mode at a resolution of 70,000 and subsequent data-dependent acquisition (dd-MS2) confirmation mode (resolution 17,500, isolation window 3.0 m/z, isolation offset 1.0 m/z, normal collision energies 17.5, 35.0, 52.5 eV), according to an inclusion list of approximately 2000 substances of toxicological interest.

Further LC-HRAM-Orbitrap-MS analyses were performed in dd-MS2 confirmation mode, according to an inclusion list of exact mass values corresponding to the MH+ ionic species of carbofuran and expected phase I and phase II metabolites, inferred from the relevant scientific literature.

Carbofuran and 3-hydroxycarbofuran certified analytical standards were used for comparative analysis.

Results & Discussion: Toxicological screening by immunoassay was negative for patient 1M, positive for cannabinoids for 2F and 4M, positive for cocaine, opiates and cannabinoids for 3M. Immunoassay results were confirmed by LC-HRAM-Orbitrap-MS.

HS-GC-MS analyses were negative for alcohol in all patients (except 2F, blood 0.30 g/L), as well as for other volatiles, including methanol.

The above aside, traditional illicit drugs were absent in all patients, as well as new psychoactive substances (NPS) and pharmaceuticals, except for medications administered in hospital, including those for intubation (ketamine, midazolam, lidocaine, fentanyl, diazepam, flumazenil).

LC-HRAM-Orbitrap-MS screening analyses indicated the possible presence of carbofuran and main metabolite 3-hydroxycarbofuran in 1M and 2F blood and urine samples. Thus, samples of all patients were subjected to further LC-HRAM-Orbitrap-MS analyses, searching for the presence of carbofuran and a complete panel of its phase I and phase II metabolites. They were identified in blood, urine, and gastric content samples of all patients, with different abundances according to individual characteristics and sample collection times. Phase I metabolites were 3-hydroxy-carbofuran, 3-keto-carbofuran, and 7-phenol-carbofuran. Phase II metabolites were 3-hydroxy-carbofuran-glucuronides and 7-phenol-carbofuran-glucuronide. Identification of metabolites was based on evaluation of their chromatographic behaviour compared to the parent compounds, accurate mass measurements of their MH+ ions in full scan conditions, evaluation of their MH+ isotopic patterns, accurate mass measurements of MH+ collision-induced product ions. In particular, the 3-hydroxy-carbofuran-glucuronides and 7-phenol-carbofuran-glucuronide were identified due to characteristic fragmentation patterns matching those of the phase I metabolites from which they derived.

Interestingly, 3-hydroxy-carbofuran only, at very low levels, was found in the urine of patient 4M, who, according to witnesses, had only taken a sip of a drink, and whose samples were taken on the third day of hospitalisation. Overall toxicology results were consistent with the clinical pictures of the patients, who were discharged 4 to 10 days later.

Conclusion: Carbofuran and many phase I and II metabolites were detected by LC-HRAM-Orbitrap-MS in several biological matrices in a case of non-lethal intoxication involving several people. This demonstrates once again that high-resolution MS techniques are excellent analytical tools for clinical and forensic toxicology applications. They allow not only highly specific and sensitive untargeted or targeted identification of individual drugs in biological fluids, but also their comprehensive and characteristic metabolite profiles, even in the absence of analytical standards, leading to highly reliable results.

CT P-11

Transmission of Per- and Polyfluoroalkyl substances (PFAS) from mother to child: analysis of maternal blood, placenta and cord blood.

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Background & Aims: Per- and polyfluoroalkyl substances (PFAS) are anthropogenic contaminants globally present in the environment. The main pathway of exposure in humans is the consumption of contaminated food and water. They are considered multi-system toxicants affecting thyroid functions, immune system, kidney and reproductive system in adults. Exposition during pregnancy is related to specific pathologies, such as preclampsia, diabetes and reduced weight at birth (IUGR). The aim of the present research was to 1) study the concentration of PFAS in blood and placenta samples; 2) evaluate the permeability of the placenta to PFAS; 3) correlate the health status of the mother during pregnancy with PFAS presence; 4) assess data on food and water consumption in relation to PFAS. The selected population was not from highly contaminated sites.

Methods: We collected blood samples from mothers and newborns and both decidua (outer side) and villi (inner side) samples after delivery. A previously developed and validated liquid chromatography coupled to high resolution accurate mass spectrometry (LC-HRAMS) method was applied for the determination of 20 PFAS, including legacy, emerging and precursors compounds. Samples were homogenised, extracted in acetonitrile and purified by solid-phase extraction (SPE) by using weak anion exchange cartridges. For quantification the isotopic dilution method was applied. Anamnestic data on maternal lifestyle and health during pregnancy were also collected through surveys and consultation of medical records.

Results & Discussion: A total of 64 samples from 16 subjects were collected. Results demonstrated PFAS presence in all the analysed samples, confirming the ubiquitarian nature of their diffusion. The most frequently detected compounds in maternal blood were PFBS/PFHxA/PFHxS/PFOA/PFOS/PFNA/PFUnA and PFPeA, while in cord blood were PFBS/PFHxA/PFHxS/PFOA/PFOS. Maternal blood showed higher concentration of total PFSA compared to cord blood, however short-chain molecules (C< 8) displayed similar concentration between maternal and cord blood, demonstrating higher permeability compared to C8 counterparts (PFOS/PFOA). PFOS and PFOA were mostly concentrated in maternal blood and in the inner part of the placenta. Placenta samples also displayed a different accumulation pattern between outer and inner side, with perfluorinated sulfonic acids more concentrated in the outer side. Data concerning water and fish consumption were also evaluated; women consuming tap water and fish more than once per week showed higher levels of PFAS in blood. Hypothyroidism and gestational diabetes, pathologies associated to PFAS exposure, were diagnosed during pregnancy in our population, with average total PFAS of 2.1 (DS 1.2) ng/ml. In healthy womens average total PFAS was 1.72 (DS 1.3) ng/ml.

Conclusion: Placenta confirmed its permeability to PFAS, but the observed different partitioning suggests that placental tissues may mediate PFAS transfer not equally. Our study contributes to understand PFAS burden in biological human samples in not highly exposed population and may help in the definition of safety limits for human health.

CT P-12

Interpreting mono- and poly-SCRA intoxications from an activity-based point of view: JWH-018 equivalents in serum as a comparative measure

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Background & Aims: Synthetic cannabinoid receptor agonists (SCRAs) are one of the largest New Psychoactive Substance classes, with more than 222 individual compounds in Europe monitored by the European Early Warning System. These compounds mimic the effects of cannabis and often surpass the effects of Δ9-THC. Due to the vast number of new compounds appearing on the recreational drug market, often very little is known about their toxicity and pharmacology at the time of their emergence. This renders the assessment of acute SCRA intoxications difficult for medical personnel as well as forensic toxicologists. Further, identification and quantification of these drugs to evaluate the extent of SCRA intoxication poses a problem.

The aim of the present study was to assess a novel comparative measure for SCRA intoxications based on the ex vivo cannabinoid (CB1) activity in serum extracts versus serum concentrations of specific SCRAs. The proposed JWH-018 equivalent entails referral to the concentration of a known reference SCRA that exerts a similar extent of cannabinoid activity as the (unknown) SCRA(s) in the serum.

Methods: This study included specific intoxication cases from a prospective observational study of patients treated in the emergency room after the consumption of a new psychoactive substance (NPS). Around 250 German emergency rooms forwarded cases to the Poisoning Information Centre Freiburg and 48 non-fatal intoxication cases were included in the present study. The following inclusion criteria were applied: i) confirmed SCRA use via liquid chromatography – tandem mass spectrometry (LC-MS/MS) and ii) sufficient sample volume for additional bioassay investigation. The presence of other drugs was not an exclusion criteria.

SCRA serum levels were quantified using LC-MS/MS in multi reaction monitoring mode. The SCRA ex vivo CB1 activity in serum extracts was assessed using an untargeted cell-based b-arrestin2 recruitment bioassay. Simultaneously, JWH-018 calibration curves were measured for the determination of activity equivalents, allowing a conversion from different SCRA serum concentrations to a JWH-018 equivalent concentration based on equal cannabinoid activity in the bioassay.

Results & Discussion: The 48 included cases comprised both mono-SCRA (n=34) and poly-SCRA intoxications (n=14). Other detected drugs were THC (2.4-25ng/mL) and its metabolites (n=12), various first-aid drugs (lidocain, pipamperone, rocuronium), MDMA (n=2, 0.31-35ng/mL), benzodiazepines (n=6, <10-150ng/mL), cathinones (n=3, 0.55-320ng/mL) and amphetamine (n=2, 7.2-82ng/mL). Twelve individual SCRAs were quantified: 5F-ADB (n=14, 0.4-9.7ng/mL), MDMB-CHMICA (n=12, 0.04-10.0ng/mL), MDMB-4en-PINACA (n=10, 0.1-0.56ng/mL), ADB-CHMINACA (n=8, 0.23-7.80ng/mL), 5F-MDMB-PICA (n=6, 0.1-45ng/mL), AB-CHMINACA (n=5, 7.3-23ng/mL), AB-FUBINACA (n=3, 0.63-4.5ng/mL), AMB-CHMICA (n=1, 0.41ng/mL), 5F-AKB-48 (n=1, 2.48ng/mL), Cumyl-PEGACLONE (n=1, 1.2ng/mL), 4F-MDMB-BICA (n=1, 5.2ng/mL) and AB-PINACA (n=1, 0.33ng/mL). Two SCRAs, MDMB-4en-PINACA and 5F-MDMB-PICA, were detected below their lower limit of quantification.

Ex vivo cannabinoid activity was measured in the sample extracts and JWH-018 equivalents could be established for 30 samples, covering a theoretical JWH-018 concentration range of 3.6-1048 ng/mL. Two samples depicted pronounced CB1 activity, with signals exceeding that of the fitted calibration curve (>5000 ng/mL). Eleven samples showed CB1 activity, but were below the lowest point of the JWH-018 calibration curve (< 2.5 ng/mL). In five samples no activity was detected.

The determined JWH-018 concentration equivalents were related to both the concentration of the parent compound (i.e. higher SCRA concentrations result in higher activity equivalents) as well as the intrinsic activation potential relative to the reference JWH-018 (stronger active compounds result in higher JWH-018 equivalent concentrations). Furthermore, the relation between JWH-018 equivalents in serum extracts and the extent of intoxication and the clinical presentation was evaluated. The severity of the intoxication with the respective SCRA was assessed based on reported symptoms and follow-up information using the Poison Severity Score (PSS). The intoxications were assigned a PSS ranging from 1 (minor), 2 (moderate), to 3 (severe). A (partial) relationship was discovered for the JWH-018 equivalent and the PSS and the causality of the SCRA for the acute drug intoxication. JWH-018 equivalents above tentative thresholds were associated with moderate/severe intoxications (> 24 ng/mL JWH-018, PSS > 1) and certain causality of the SCRA to the acute drug intoxications (> 47 ng/mL JWH-018). JWH-018 equivalents below these thresholds were associated with PSS of 1-3 and both probable and certain causality. Additionally, a high variety of toxicological symptoms was recorded for the included cases and no evident relationship to the activity in serum could be established.

Conclusion: We propose the novel approach of using a 'JWH-018 concentration equivalent' as a comparative measure to study mono- and poly-SCRA intoxications from a cannabinoid activity point-of-view.

Activity equivalent determination as a form of untargeted screening can aid in the identification of SCRA intoxications as well as in the evaluation of the extent of an intoxication, without prior knowledge on the individual SCRA's pharmacological profile or harm potential. This holds especially true for novel emerging SCRAS which have not yet been identified or characterized, or for which no reference compound is available yet.

Retrospective analysis of cannabis testing outcomes in the emergency department and intensive care unit at Saadna Abdennour University Hospital, Setif, Algeria: Insights from the toxicology laboratory

CT P-13

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Background & Aims: Cannabis stands as the most widely used illicit drug, cultivated, and trafficked globally. In concordance with numerous nations worldwide, Algeria has witnessed a surge in cannabis seizures. The illicit nature of cannabis in Algeria often leads patients to withhold their cannabis intake during emergency department visits, potentially resulting in an underestimation of drug exposure. Consequently, toxicological analysis becomes imperative, particularly in cases where patients present symptoms suggestive of acute cannabis poisoning, which is acknowledged for inducing diverse symptoms encompassing cardiovascular and neurological manifestations, occasionally necessitating admission to the intensive care unit. The aim of our study is to elucidate the outcomes of cannabis testing in response to toxicological analysis requests from the emergency department and intensive care unit at Saadna Abdennour University Hospital in Setif, Algeria. Furthermore, we seek to delineate the demographic and clinical characteristics of individuals with confirmed cannabis usage through positive test results.

Methods: A retrospective analysis was conducted utilizing data from the emergency toxicology unit over the preceding five years, encompassing analysis requests from the Emergency Department (ED) and Intensive Care Unit (ICU). Toxicological testing for cannabis in urine was performed using one of two qualitative analytical techniques: enzyme multiplied immunotechnic (Seimens Viva Pro-E®) or immunochromatography rapid test (Syva Rapid® and Narcotest®), with a limit of detection of 50ng/ml. Statistical analysis was conducted using the chi-square test and Fisher's exact test in R software, considering a p-value<0.05 as statistically significant.

Results & Discussion: Over the course of five years, 188 cannabis tests were conducted, with 124 (66%) originating from the emergency department and 64 (34%) from the intensive care unit. Twenty-one (11%) of these tests yielded positive results, with no statistically significant difference observed between the positive results from the intensive care unit and emergency department (p>0.05). Among the 124 cannabis tests from the emergency department, ten (8%) were positive, with one patient requiring endotracheal intubation and mechanical ventilation. In the intensive care unit, 64 tests were conducted, with 11 (17%) yielding positive results, three of whom required endotracheal intubation and mechanical ventilation. Positive cannabis results were associated with positive results for other illicit drugs in 33% of cases, a statistically significant association (p<0.05). In addition to illicit drugs, psychiatric medications were detected alongside cannabis and illicit drugs in patients admitted to the emergency department and intensive care unit, primarily benzodiazepines, antidepressants, neuroleptics, and antiepileptic drugs (phenobarbital, carbamazepine). However, the presence of positive results in urine tests does not automatically indicate recent consumption. Male patients constituted 95% of positive cannabis results (p<0.05), with 85% of patients with positive results being over 18 years old (p<0.05). Neurological symptoms were the predominant findings in patients with positive cannabis results, although this observation was not statistically significant. The presence of correlation between cannabis use and neurological symptoms does not inherently imply causation.

Conclusion: The retrospective analysis of cannabis testing outcomes in the emergency department and intensive care unit at Toxicology laboratory of Saadna Abdennour University Hospital, Setif, Algeria, provides valuable insights into the prevalence and correlates of cannabis usage among patients admitted to emergency department and intensive care unit. The findings underscore the importance of toxicological analysis in identifying cannabis exposure, particularly in patients with severe clinical manifestations necessitating intensive care intervention. Moreover, the significant association between positive cannabis results and other illicit drugs highlights the complexity of substance use patterns among the population under study. Further research is warranted to explore the implications of cannabis usage and admissions to ICU and emergency department.

CT P-14

Determination of benzoylecgonine below the cut-off of immunochromatographic tests in pediatric population by GC/MS

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Background & Aims: Cocaine consumption has remained stable in the Uruguayan population for at least 10 years, being the fifth most consumed drug in the country with a lifetime prevalence of 7.8% (approximately 139,000 people), after the consumption of alcohol, tobacco, tranquilizers and cannabis (VII National Survey on drug use in the general population, 2019). Children of cocaine-using mothers are exposed prenatally and during breastfeeding. They also present a risk of unintentional ingestion due to direct contact with the substance. The symptoms that can be observed are seizures, vomiting, irritability, psychomotor excitement and tachycardia, among others. Rapid screening methods to detect the presence of the drug are immunochromatographic tests that are manufactured primarily for the adult population. Accordingly, the cut offs they present are generally 300 to 150ng/mL of benzoylecgonine for screening purposes and 100 to 50ng/mL for confirmatory purposes. These concentrations are above

the intoxication values found in the pediatric population. Therefore, the objective of this work was to implement a method for the detection and quantification of one of the metabolites of cocaine, benzoylecgonine, in urine samples from pediatric patients by isotopic dilution by gas chromatography coupled to a mass spectrometer (GC/MS).

Methods: Fifteen urine samples from pediatric patients under 6 years old who consulted at the Department of Toxicology of the Faculty of Medicine were analyzed. All children presented clinical suspicion and a favorable context that was consistent with possible unintencional cocaine intoxication, but presented negative immunochromatographic tests performed in emergency services. The collected urine was spiked with the internal standard Benzoylecgonine-D3 at a concentration of 15 ng/mL. The technique includes a liquid-liquid extraction (LLE) and subsequent derivatization of the samples with BSTFA prior to injection into the GC/MS.

Results & Discussion: Analysis of the 15 urine samples revealed the presence of benzoylecgonine in 11 samples, all below the cut-off limits of standard immunochromatographic screening tests. Nine samples exhibited concentrations of benzoylecgnonine below the confirmatory limit proposed for adults (100 ng/mL), ranging from 1 to 90 ng/mL. These findings underscore the inadequacy of applying adult-based cut-off limits to pediatric populations and highlight the need for tailored detection methods.

Conclusion: It was possible to implement a confirmation method that allows detecting and quantifying one of the cocaine metabolites in pediatric patients with suspected intoxication, who often present negative results in immunochromatographic tests because they present concentrations below the cut-off limits used for the adult population. For this reason, the limits proposed for the adult population should not be applied to the pediatric population. Although pediatric cocaine exposures are rare, these findings show us that we may underestimate cases of pediatric cocaine poisoning that result in more severe outcomes than most unintentional pediatric poisonings.

Comparison between drugs of abuse testing by immunoassay and a commercial LC-MS/MS method in urine samples from patients in opiates maintenance therapy

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Background & Aims: Screening for drugs of abuse with immunoassay in urine samples from patients in heroin substitution treatment can still be regarded as standard practice. However, for an increasing number of drugs no homogenous immunoassays are available. In this study we therefore compare our routine immunoassay screening panel with a ß-test version of a commercial LC-MS/MS kit (Chromsystems) targeting 108 substances.

Methods: Consecutive 507 routine urine samples from 507 patients (99 female) substituted with Methadone/L-Polamidon, Buprenorphine or Morphine were analyzed with our standard immunoassay panel on an AU 680 (Beckman Coulter). The following CEDIAs (Thermo Fisher) were included (cutoff in ng/mL): Amphetamines (500), Barbiturates (100), Benzodiazepines/online hydrolysis (100), Buprenorphine (2), Cocaine (50), EDDP (100), THC (25), LSD (0.5). The opiates assay (100) was a DRI (Thermo Fisher) and the pregabalin assay (200) was from Specialty Diagnostics. Every sample was analyzed with the Chromsystems method on a Waters Acquity/Xevo-XS according to the kit instructions. The detector operated in positive mode (barbiturates in negative mode) and 2 or 3 transitions were recorded for analytes and internal standards. Runtime was 12.5 minutes. Urine calibrators (6-point), 3 control levels and a hydrolysis control sample were included in the kit and ran with every series. Cutoffs (ng/mL) were set slightly above the lowest calibrator: amphetamines/stimulants (n = 17): 30, barbiturates (n = 10): 100, benzodiazepines (n = 31): 30, cocaine (n = 4): 20, THC-COOH (n = 1): 7.5, opiates/opioids (n = 31): 1-30, Z-substances (n = 3): 30, pregabalin (n = 1): 30, LSD (n = 2): 0.5, others (n = 8): 10 – 30. Discrepant samples were further analyzed with inhouse MS methods.

Results & Discussion: Amphetamines: 31 samples were positive in the Amphetamines-CEDIA from which 21 could be confirmed with the Chromsystems method (amphetamine: 21, methamphetamine: 8, MDMA: 1, MDA: 1). Six samples were identified false positive with our inhouse methods: m-CPP and trazodone, bupropion (2) and cyclohexylamine (3). For 4 samples no explanation was found. The Chromsystems method found 16 additional samples positive for amphetamines at cutoff 30 ng/mL (amphetamine: 15, methamphetamine: 2, MDMA: 1). Barbiturates: 2 samples were positive in the Barbiturates-CEDIA and confirmed by the detection of phenobarbital. Benzodiazepines: 90 samples were positive in the Benzodiazepines-CEDIA from which 87 were confirmed with the Chromsystems method (nordiazepam: 47, oxazepam: 57, temazepam: 51, clonazepam: 8, 7-aminoclonazepam: 51, Alprazolam: 9, 7-OH-Alprazolam: 13, Lorazepam: 10, 3-OH-Bromazepam: 2). For three samples no cross-reacting substance was identified. The Chromsystems method found 4 additional samples positive for benzodiazepines at cutoff 30 ng/mL (7-aminoclonazepam: 3, Oxazepam: 1). Buprenorphine: 163 samples were positive in the Buprenorphine-CEDIA from which 111 were confirmed with the Chromsystems method (buprenorphine: 111, norbuprenorphine: 105). The 6 samples which did not contain norbuprenorphine were negative for the corresponding

glucuronides with inhouse methods indicating sample contamination. Forty-six samples were identified false positive due to very high morphine concentrations. For 6 samples no explanation was found. The Chromsystems method found 4 additional samples positive for buprenorphine at cutoff 1 ng/mL with no norbuprenorphine. Cocaine: 80 samples were positive in the Cocaine-CEDIA which all were confirmed with the Chromsystems method (benzoylecgonine: 80, cocaine: 45, norcocaine: 24, cocaethylene: 11). The Chromsystems method found 15 additional samples positive for cocaine metabolites at cutoff 20 ng/mL (benzoylecgonine: 15, cocaine: 1). EDDP: 314 samples were positive in the EDDP-CEDIA which all were confirmed with the Chromsystems method. THC: 217 samples were positive in the THC-CEDIA from which 213 were confirmed with the Chromsystems method. Three samples were identified false positive with our inhouse method by detecting HHC-COOH. For 1 sample no explanation was found. The Chromsystems method found 4 additional samples positive for THC-COOH at cutoff 7.5 ng/mL. LSD: 13 samples were positive in the LSD-CEDIA. All samples were identified as false positive due to cross-reactivity with fentanyl and norfentanyl (10) or ambroxol (3, inhouse method). Opiates: 124 samples were positive in the Opiates-DRI from which 119 were confirmed with the Chromsystems method (morphine: 119, 6-acetylmorphne: 26, codeine: 83, 6-acetylcodeine: 13, norcodeine: 28, hydromorphone: 97, hydrocodone: 2). Two samples were identified false positive due to ofloxacin with our inhouse methods. Two samples contained codeine-glucuronide which had not been fully hydrolyzed. For 1 sample no explanation was found. Pregabalin: 87 samples were positive in the pregabalin-assay which were all confirmed with the Chromsystems method. The Chromsystems method found 4 additional samples positive at cutoff 30 ng/mL. Other substances: 11 different additional drugs and/or their metabolites were detected, e.g.: methylphenidate (6), fentanyl (14), tilidine (1), tramadol (3), tapentadol (1), gabapentin (3), promethazine (7), quetiapine (28).

Conclusion: LC-MS/MS screening reveals several advantages compared to immunoassay screening: 1. LC-MS/MS screening has no false positives, 2. higher sensitivity increases positive rate, 3. targeting metabolites proves body passage, 4. quantification allows follow-up monitoring, 5. additional substances are found.

CT P-16

Urine opiates test results after consumption of flapjacks containing codeine-predominant poppy seeds

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Background & Aims: It is common knowledge that poppy seeds food products may be contaminated with the poppy latex and therefore can contain opium alkaloids with morphine predominating. Consumption of these products can cause a positive urine opiates test result. Confirmation analysis normally detects morphine and often but in much lower concentration codeine is found. During the last months we received several clinical urine samples with a high codeine to morphine ratio. This could be interpreted as a recent ingestion of codeine. However, one patient denied intake of codeine and proposed that flapjacks from the hospital shop containing poppy seeds were the cause. In this study we analysed different lots of the corresponding flapjacks for their morphine and codeine content and the urine samples from volunteers who consumed them.

Methods: From 32 "Hafervoll – Organic-Flapjack – POPPY SEED & APRICOT" (weight: mean = 68.3 g, CV = 5.1%, median = 67.9 g) a thin middle segment was cut out (mean = 222 mg, CV = 7.8%, median = 218 mg) and homogenized with yttrium coated ceramic balls in a high frequency shaker (Bead Ruptor Elite, Omni). The samples were further analysed for morphine and codeine with UPLC-MS/MS. The remaining amount was eaten by 32 colleagues from the lab and all urine samples from the next 1- 2 days were collected (range first sample: 0.17 to 8.92 h, median = 2.47 h, range last sample: 22.2 to 49.0 h, median = 34.8 h). The flapjacks were from different shops and different lots: 10x 22126, 6x 22227, 3x 22228 and 13x 23010. Every urine sample (n = 309, 5 to 12 samples/individual) was tested for opiates (cutoff 100 ng/mL, DRI, Thermo Fisher) and creatinine (Thermo Fisher) on an AU 680 clinical chemistry analyser (Beckman Coulter). Quantitative analysis for morphine and codeine in all urine samples was performed after enzymatic hydrolysis (B-One, Kura Biotech) on a Waters Acquity/Xevo-XS tandem mass-spectrometer. The detector operated in positive mode and 3 and 2 transitions were recorded for analytes and internal standards. Lower limits of quantification were at least 2 ng/mL. The method is accredited according to forensic standards.

Results & Discussion: The flapjacks from the hospital shop (lot 22126, n = 10) turned out to contain high amounts of codeine: range 7106 to 48489 ng/g, mean = 19040 ng/g and median = 12514 ng/g. The morphine concentrations were much lower: 18.4 to 97.5 ng/g, median = 41.2 ng/g. The highest urine codeine concentrations were reached between 3.8 to 6.42 h and ranged from 1048 to 6925 ng/mL (median = 3187 ng/mL). The highest urine morphine concentrations were reached between 3.8 to 10.75 h and ranged from 25.2 to 1032 ng/mL (median = 91.6 ng/mL). These samples were all above the upper measuring range of the immunoassay (>2000 ng/mL). One individual turned out as fast metabolizer

of codeine to morphine. At cutoff 100 ng/mL the time point until the first sample was negative ranged from 21.0 to 38.1 h (median = 31.8 h). The flapjacks from the other shops (lots 22227, 22228, 23010, n = 22) revealed much lower codeine concentrations: range 0.6 to 48.8 ng/g, mean = 5.07 ng/g and median = 2.75 ng/g. The morphine concentrations were higher and ranged from 6.4 to 70.1 ng/g, median = 27.1 ng/g. From these urine samples only one sample was above the immunoassay cutoff. However, with UPLC-MS/MS morphine could be detected at low concentrations in 21 individuals between 11.0 and 37.4 h after consumption of the flapjack. Codeine was detectable between 2.25 and 33.8 h at low concentrations in 13 individuals only.

Conclusion: Food stuff products may contain a poppy seeds variation contaminated with high amounts of codeine. Ingestion of these products can produce opiates-positive urine test results. The confirmation analysis will result in high codeine to morphine ratios which could suggest a recent codeine intake.

Gonadotoxic effects of tobacco on human seminal fluid

CT P-17

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Background & Aims: According to World Health Organization report (2019), prevalence of tobacco smoking remains high worldwide, with an estimated 19% of the adult population is using tobacco products: 33% among males and 6% among females. Consistently increasing abuse of tobacco is of alarming concern since tobacco use is unequivocally associated with multiple chronic disorders and adverse health outcomes including potential gonadotoxic effects in both men and women. Evidence suggests that tobacco affects reproductive health more than alcohol. Tobacco consumption yields approximately 4000 compounds. Tobacco smoke can be divided into a gaseous phase and a particulate phase. The principal harmful components of gaseous phase are CO2, nitrogen oxide, ammonia and volatile hydrocarbons whereas nicotine is the main component of the particulate phase. Nicotine is a natural, volatile, colorless, water-soluble alkaloid. Pharmacological action of nicotine takes place at the autonomic ganglia. Nicotine has dual action i.e.; it acts as CNS stimulant as well as depressant. Nicotine is quickly absorbed through the respiratory tract, mouth, mucosa and skin. It is mainly metabolized into cotinine majorly by liver but also by kidney and lung. Nicotine and cotinine levels have not only been detected in urine, serum, saliva, hair and milk but also in seminal plasma at significant levels.

The purpose of the study is to determine the potential gonadotoxic effects of tobacco abuse on semen quality, seminal plasma antioxidant activity and sperm DNA integrity and to determine relative concentrations of nicotine and cotinine in human seminal fluid compared to urine and serum.

Methods: The study included 200 male participants aged 20–40 years. The study requirements were carefully explained to participants, who provided written informed consent before entering the study. The study was conducted in accordance with the Declaration of Helsinki. Urine, serum and semen samples were obtained from cigarette smokers (having history of cigarette smoking of at least one year). While the inclusion criteria were experimental subjects with azoospermia, exposure to chemicals, alcohol consumption and subjects with a history of recreational drug abuse. Seminal parameters were evaluated as per WHO guidelines. Quantification of nicotine and cotinine in urine, serum and semen samples was done by Ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS-MS). TUNEL assay (Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-nick end labeling) was used for DNA Fragmentation Index. The TUNEL assay detected DNA fragmentation, indicating apoptosis or damage, by labeling DNA ends with fluorescent or enzymatic markers. Terminal deoxynucleotidyl transferase (TdT) incorporated labeled nucleotides into DNA breaks or nicks. Fragmented DNA ends were evaluated via flow cytometry, providing insights into DNA quality. Minimal fragmentation is characteristic of high-quality DNA, reflecting intact genomic integrity, while heightened fragmentation suggests compromised DNA quality.

Results & Discussion: In all cases, the corresponding semen sample was positive for nicotine and cotinine ranging from 5 - 50 ng/mL for nicotine and 5 - 800 ng/mL for cotinine. Nicotine and cotinine were detected in human seminal plasma at concentrations as high as in plasma. Urinary cotinine concentrations (exceeding 1000 ng/mL) were highly correlated with serum concentrations. A significant decrease in semen quality (sperm count and sperm progressive motility etc.) was found for cigarette smokers ($p < 0.05$). DNA fragmentation index was significantly higher for smokers ($p < 0.05$). Nicotine and cotinine also inhibited catalase and glutathione reductase activity, contributing to an accumulation of reactive oxygen species.

Conclusion: The present study suggests that tobacco smoking significantly affects human seminal parameters, seminal plasma antioxidant activity and sperm DNA integrity. It is therefore recommended that male smokers should stop smoking tobacco well in advance of planning to have children.

CT P–18

Accidental cannabis oil intoxication in a pediatric patient: A case report

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Background & Aims: The prevalence of medicinal cannabis usage is escalating among adults, driven by recent therapeutic advancements across various health conditions. This trend is extending to pediatric patients, particularly for conditions like epilepsy, autism spectrum disorder (ASD), and attention deficit hyperactivity disorder (ADHD). Despite its increasing use, there's limited data on the safety profile for pediatric patients under specific exposure conditions. This study aims to report a case of accidental cannabis oil exposure in a pediatric patient undergoing ASD treatment, managed by the Campinas Poison Control Center. A nine-year-old female patient, weighing 40 kg, diagnosed with ASD, inadvertently ingested approximately 30 mL of commercially available cannabis oil, containing cannabidiol (CBD) and tetrahydrocannabinol (THC) in a 1:1 ratio (10 mg/mL). She had been using this product therapeutically for three years, prescribed and legally imported following Brazilian regulations (RDC n° 660/2022 ANVISA). Following ingestion, the patient rapidly exhibited significant drowsiness (Glasgow 14) and was promptly referred to a specialized emergency unit. Upon admission, she was unresponsive and developed hypotension (systolic blood pressure 70–90 mmHg and diastolic blood pressure 40–50 mmHg) and lethargy, persisting for 12 hours. Biochemical tests, including complete blood count, renal, and liver function, showed no abnormalities. Symptomatic and supportive treatment ensued, with the child remaining hospitalized in the intensive care unit for 24 hours until complete resolution of symptoms before discharge.

Methods: Blood and urine samples were collected during medical care for laboratory diagnostics, along with an oil aliquot for subsequent compositional analysis. Liquid chromatography–tandem mass spectrometry (LC-MS/MS) was used for blood and urine analyses, while high-performance liquid chromatography with diode array detection (HPLC-DAD) analyzed the cannabis oil. Post-discharge, healthcare professionals reviewed the case, classifying intoxication severity using the Poisoning Severity Score (PSS).

Results & Discussion: Initial calculation of the ingested dose (ID) of CBD and THC was 7.5 mg/kg based on admission data. However, HPLC-DAD analysis revealed CBD and THC concentrations of 8 and 7 mg/mL, respectively, leading to a recalculated ID of 6 and 5.25 mg/kg. Blood analysis by LC-MS/MS showed elevated CBD and THC concentrations (> 10 ng/mL) along with related metabolites, while urine analysis confirmed exposure only to CBD and THC, without other psychoactive substances. The clinical presentation, symptoms, and response to treatment were classified as minor intoxication (PSS 1). This case underscores the significant health implications of accidental exposure to controlled medications in children. Although the patient had been using the product therapeutically, accidental overdose resulted in notable symptoms, necessitating intensive care monitoring. The positive response to supportive measures underscores the importance of timely intervention. CBD and THC act on endocannabinoid receptors, and their ingestion in high doses can potentiate cardiovascular and sleep cycle effects, explaining the observed symptoms. While there's no specific antidote for cannabinoid intoxication, supportive care remains pivotal. This case highlights the exclusive role of CBD and THC in the observed effects, emphasizing the importance of laboratory confirmation and parental reporting for targeted therapy. However, in scenarios lacking such information, clinical investigation may be prolonged, considering the varied etiologies of presenting symptoms. In Brazil, the legislation that defines the criteria and procedures for the importation for personal use for health treatment of Cannabis-based products is still very recently implemented, which means that patients do not take due care with the storage and handling of these products. Despite being a low toxicity product, Cannabis oil remains a medicine that requires the establishment of care measures, such as controlling access for children. Therefore, this work plays an important role in alerting the population and health professionals about the relevance of care with this type of medication, through a case report of an accidental poisoning, in order to reduce damages, such as possible hospitalizations in emergency units in critical cases.

Conclusion: With the escalating prescriptions for cannabis oil, healthcare professionals need readiness to diagnose and manage such cases, alongside educating patients about potential mishandling risks leading to overdose. Developing therapeutic strategies to mitigate potential harm is imperative. Furthermore, given the growing use of medicinal cannabis in Brazil, stringent quality control of these products, employing validated analytical techniques, is crucial to ensure alignment between reported label information and actual product composition and quality. The present work was supported by funding from the Pharmaceutical Security Nucleus Project, object of Agreement n° 895688/2019, the result of a partnership between the Ministry of Justice and Public Security of Brazil, through the Fund for the Defense of Diffuse Rights and the State University of Campinas.

Norfludiazepam: a case of non-fatal acute poisoning

CT P-19

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Background & Aims: Norfludiazepam (also known as norflurazepam or N-dealkylflurazepam) is a synthetic benzodiazepine with a long half-life that appeared on the underground market in 2017 [New Benzodiazepines in Europe – review. EMCDDA]; it is also the metabolite of several benzodiazepines marketed in Europe (midazolam, quetiazepam, flurazepam). To the authors knowledge, no case of poisoning have been previously described with this compound. Here is reported a case of acute intoxication, from unknown origin, with norfludiazepam.

Methods: Three blood samples were drawn during hospitalization for toxicological analysis: D0, D+2 and D+5. For each blood sample were performed i) an immunological screening for acetaminophen, tricyclic antidepressants and benzodiazepines using an EMIT test (Atellica®, Siemens), and ii) a non-targeted toxicological screening by LC-HRMS (Orbitrap Exploris 120 Mass Spectrometer, ThermoFisher Scientific) using a previously published method [Becam et al. J Chromatogr B Analyt Technol Biomed Life Sci 2023;30:1224:123739]. Norfludiazepam, hydroxyzine and cetirizine were quantified using the same analytical method.

Results & Discussion: The patient was a 68-years-old woman with a history of acute idiopathic necrotizing pancreatitis and suffering from overweight (1.57 m, 71.5 kg). Her husband called for help because she has been drowsy since waking up. On arrival at the emergency, the patient's oxygen saturation was 90%. She was put on 3L of oxygen. Her Glasgow Coma Score (GCS) was 10, with bilateral miosis. No motor abnormalities were found, but she was unable to perform the Mingazzini maneuver, indicating overall muscle weakness. Further clinical examinations revealed no cerebral abnormalities. A 0.5mg flumazenil test was carried out, enabling the patient to go from a GCS = 10 to 14, suggesting a benzodiazepine intoxication. She was transferred to the internal medicine department and remained hospitalized for 6 days. During her hospitalization, she remained dependent to oxygen (2L of O2). A psychiatric consultation was carried out, the psychiatrist did not diagnose any signs of major clinical decompensation (anxiety, schizophrenia, mood disorder, etc.), and the suicidal risk was considered low. On discharge from hospital, the patient was suffering from anterograde amnesia. Unable to explain this acute intoxication, the medical team advised her to lodge a complaint, but she never did it.

EMIT screening results were positive for benzodiazepines in all 3 samples (D0, D+2 and D+5). Toxicological screening by LC-HRMS showed the presence of toxic concentrations of hydroxyzine at D0 (D0 =245 ng/mL, D+2=60 ng/mL, D+5= 9 ng/mL), as well as the presence of its main metabolite, cetirizine (D0=700 ng/mL, D+2=147 ng/mL, D+5 < 5 ng/mL). No benzodiazepines available in France were detected, but non-targeted screening showed the presence of norfludiazepam in the 3 samples at the following concentrations: D0 = 1330 ng/mL, D+2 = 945 ng/mL and D+5 = 780 ng/mL. Calculated half-life based on the elimination constant (Kel) for a monocompartmental model was 157h (D0 to D+5).

The authors present here the first case of norfludiazepam intoxication associated with hydroxyzine intoxication. Concentrations greater than 500 ng/mL for norfludiazepam may be toxic, [Clarke's Isolation and Identification of Drugs. London: Pharmaceutical Press; 1986.] A blood concentration of 770 ng/mL for norfludiazepam was observed in a case of acute poisoning to flurazepam [Vantaggiato et al. Am J Forensic Med Pathol. 2007;28:55-58]. Norfludiazepam is also the metabolite of several benzodiazepines: midazolam, quetiazepam, flurazepam; the absence of these compounds along with N1-hydroxyethylnorflurazepam in the 3 plasma samples suggests a direct absorption of the compound [Moosmann et al. Drug Test Anal. 2019;11:541–549]. Norfludiazepam elimination half-life ranges from 40h to 150h, which is close to the calculated half-life (157h) and consistent with the patient's prolonged hospitalization [Greenblatt et al. Pharmacology 1983;27(Suppl. 2):70–75]. The patient did not report having taken hydroxyzine and showed no suicidal intent, suggesting accidental poisoning, or deliberate poisoning by a third party. Following this event, during consultations with her GP, the patient stated that she had no recollection of it, and reiterated her wish not to lodge a complaint.

Conclusion: The synthetic benzodiazepine norfludiazepam can induce severe and prolonged acute poisoning. To the authors knowledge this is the first case described in the current literature.

Approval of the therapeutic use of Cannabis derivatives in Italy

CT P-20

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Background & Aims: Object of this article is to illustrate aspects of debate and controversial, related to the authorization for the therapeutic use of Cannabis derivative products in Italy, occurred with the Ministerial Decree 09/11/2015; the main focus deals with therapeutic use and limited indications by pathologies; secondly, the cultivation for personale use is considered, in regard to variable court verdicts; first, in article 1 the law authorizes the cultivation, import and export of cannabis derivatives for therapeutic purposes and the Military chemical pharmaceutical institute of Florence is designated as a suitable place for the production of these produced in accordance with the guidelines decreed by the Italian Medicines Agency (AIFA).

Methods: We took into consideration (Paragraph 4.6) the pathological conditions in which it is permitted by law to use cannabinoid oils for therapeutic purposes, and indication:
- Analgesia in pathologies involving spasticity associated with pain (multiple sclerosis, spinal cord injuries) resistant to conventional therapies
- Analgesia in chronic pain, especially in neurogenic pain in which treatment with NSAIDs has proven ineffective
- Antikinetic and antiemetic effect in nausea and vomiting induced by chemotherapy, radiotherapy, HIV therapy
- Stimulating effect in cachexia, anorexia, loss of appetite in cancer or AIDS patients and in anorexia nervosa which cannot be achieved with standard treatments
- Hypotensive effect in glaucoma resistant to conventional therapies
- Reduction of involuntary movements of the face and body in Gilles de la Tourette syndrome which cannot be achieved with standard treatments.

An updated revision of the scientific literature was performed.

Results & Discussion: In Italy, in recent years the consumption of Cannabis derivatives for therapeutic purposes has increased significantly. According to data reported by the Ministry of Health, from 2014 to 2022, the consumption of therapeutic cannabis in grams went from 58,590 in 2014 to 1,560,680 in 2022.

From a legal point of view, the cultivation of Cannabis indica that contains a concentration of active principles of THC such as to cause a narcotic and psychotropic effect is prohibited. Even the possession of even one plant of the indica species is considered a crime. Some Supreme Court rulings have established that any cultivation of hemp plants is considered a "cultivation crime", except for those provided for by law and that it is not necessary to carry out a technical assessment on the actual concentration of THC produced by the plant. Therefore, if the cultivated species belongs to one of those that have a THC concentration higher than the values permitted by law, even if once cultivated the THC does not actually reach this concentration, it is to be considered a crime. The crime of cultivation therefore occurs when the planted seed has germinated and reproduced in any soil or container. On 2 December 2016, law no. 242 was promulgated, with which the cultivation of some varieties of hemp was allowed to pursue the following purposes:
- Reduction of environmental impact
- Reduction of land consumption and desertification
- Prevent the loss of biodiversity

This law allows a THC concentration of between 0.2% and 0.6%. The seizure and destruction of hemp cultivation is possible if the THC concentration found is higher than 0.6%.

Conclusion: The topic of "Regulated versus unregulated cannabis " is still controversial within legal approach. In addition to limiting interactions with the unregulated market and with the criminal justice system, regulated cannabis is safer and more predictable. Unregulated cannabis has been found in many studies to be contaminated with harmful pesticides, heavy metals, molds, microbes, or mycotoxins. Most states with legalized cannabis test regulated cannabis for these contaminants, in addition to confirming cannabinoid dosing and labeling. However, the ways in which regulation and testing are implemented differ by Countries in EU; probably a legal harmonization in the context of unified Europe could benefit the profile of health and protection of collective safety. A constant evaluation of the therapeutic utility index is essential.

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CT P-21

Can Carboxy-THC be accurately quantified in urine using a rapid multi-analyte drugs of abuse method?

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Background & Aims: Quantification of the cannabis metabolite, THC-COOH (THC-A), in urine has presented challenges for both clinical and forensic toxicology laboratories. In order to shorten assay turn-around times, our laboratory sought to develop a rapid multi-analyte urine drugs of abuse method that removed the time-consuming hydrolysis step and instead quantified the glucuronide metabolites. One goal for the new method was to include cannabis through the quantification of THC-A and THC-A glucuronide (THC-A-gluc). However, we found that THC-A-gluc was unable to be accurately quantified in authentic patient samples and chose to instead develop a stand-alone method for cannabis quantification. An additional requirement for the method was the ability to separate Δ8 THC-A and Δ9 THC-A.

Methods: Reference materials for Δ9 THC-A-gluc, Δ8 THC-A, Δ9 THC-A, Δ9 THC-A-D3 and Δ9 THC-A-D3-gluc were purchased from Lipomed (USA) and Cerilliant (USA). The separation of Δ8 THC-A and Δ9 THC-A was investigated using a UCT Selectra DA column (50 x 2.1 mm, 3 µm) and Shim-pack Volex C18 column (50 x 2.1 mm, 2.7 µm). Extractions were performed using a Shimadzu CLAM 2030 liquid handler which involved sample dilution, filtration, incubation (if required) and direct injection onto a Shimadzu 8060NX. Sample hydrolysis was performed using 10M Potassium Hydroxide, 3 minutes incubation at 60oC followed by addition of glacial acetic acid.

Results & Discussion: THC-A-gluc was able to be accurately quantified when spiked in certified blank urine. However, when authentic patient samples with known levels of THC-A were tested (previously analysed using a validated method with hydrolysis), the levels of THC-A-gluc did not match the known levels. Further investigation showed the presence of at least two isomers of THC-A-gluc in spiked blank urine and several isomers in authentic patient samples. The presence of two isomers in spiked urine may be due to the certified reference material containing the racemic mix of THC-A-Gluc, however the ratio of the isomers differed between spiked samples, this indicated that the THC-A-gluc compound was being altered by the urine samples. In authentic patient samples (from individuals who had used cannabis) multiple chromatographically separated peaks with the same parent mass and at least one product ion were identified, a phenomenon previously reported by Hanisch et al., 2017. It was determined that the quantification of THC-A without hydrolysis was not viable and THC-A-gluc could not be added to the laboratory's multi-analyte method. As such, we developed a stand-alone LC/MS/MS method for the quantification of THC-A, with the additional goal of separating the Δ8 and Δ9 THC-A variants. In recent times, the prevalence of Δ8 THC-A has been increasing, as its conversion from cannabidiol (CBD) has become more popular. As the standard used in Australia by clinical toxicology laboratories performing medico-legal and workplace drug testing on urine (AS/NZ4308) does not cover Δ8 THC-A, some laboratories have not determined if their methods can distinguish between the two variants. During method development we tested two common stationary phases, biphenyl and C18. We found that the Δ8 and Δ9 variants could not be separated by a biphenyl phase, but were able to be separated on a C18 column. This counted as an additional reason for THC-A to be removed from the multi-analyte method, which was found to have the best results when the biphenyl column was used.

Conclusion: The Toxicology laboratory at Melbourne Pathology found that the quantification of cannabis in urine could not be achieved successfully when included in a rapid multi-analyte drugs of abuse method where the hydrolysis step had been removed. A stand-alone cannabis method was developed that included hydrolysis and the separation of Δ8 and Δ9 THC-COOH.

Reference:
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Investigation of potential antitumor effects of cannabinoid type 2 receptor ligand on chronic myeloid leukemia cells, *in vitro*

CT P-22

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Background & Aims: Chronic myeloid leukemia (CML) is a myeloproliferative disorder caused by the clonal proliferation of bone marrow hematopoietic stem cells and is treated with various tyrosine kinase inhibitors (TKIs). It is known from many studies that cannabinoid receptor (CBR) ligands exhibit anti-tumor effects in various cancer types including chronic myeloid leukemia. However, the possible anti-tumor activity of the cannabinoid receptor ligand used in this study on chronic myeloid leukemia cells is unknown. Therefore, this study aims to investigate the possible antiproliferative effect of cannabinoid type 2 receptor ligand as a single agent and in combination with tyrosine kinase inhibitors imatinib and ponatinib on chronic myeloid leukemia model, K562 cells.

Methods: Colorimetric cytotoxicity methods involve measurements based on the color change of tetrazolium salts using an XTT (cell proliferation assay kit). Cytotoxicity assays were performed using the XTT method to determine

the cytotoxic effect administration of cannabinoid type 2 receptor ligand (JWH-073, Cerilliant) as a single agent and in combination with the tyrosine kinase inhibitors imatinib and ponatinib on the viability of K562 cells (K-562 are lymphoblast cells isolated from the bone marrow of a 53-year-old chronic myelogenous leukemia patient). Considering the anti-proliferative effects of cannabinoid type 2 receptor ligands on K562 cells, the new agent used in the study, cannabinoid type 2 receptor ligand, was applied to K562 cells. Subsequently, apoptosis experiments were performed using the which a protein Annexin V (an anticoagulant protein)- fluorescein isothiocyanate (FITC) and Propidium iodide (PI) which is a popular red-fluorescent nuclear and chromosome counterstain method to determine the apoptotic effect of cannabinoid type 2 receptor ligand.

The mRNA transcript levels of JAK2, STAT5A and STAT3 genes, which are key genes in the JAK/STAT signaling pathway that play a key role in the carcinogenesis process of CML, BCL-X gene associated with apoptosis, tumor suppressor TP53, an oncogene MYC and ABL1, E2F1, a gene involved in the cell cycle, and finally CNR1 and CNR2 genes, components of the endocannabinoid system, were statistically evaluated by RT-qPCR.

Results & Discussion: The IC50 value (inhibitory concentration; the dose that destroys 50% of the cells) of the cannabinoid type 2 receptor ligand on leukemic cells and the ED50 value (dose of combination) of the combination with imatinib and ponatinib were determined by XTT assays for cytotoxicity testing. The IC50 value of the cannabinoid type 2 receptor ligand (JWH-073) on K562 cells was determined to be 6.14 µM. The IC50 value of THC was 14,3 µM on HL60 (acute lymphoblastic cells), The IC50 value of THC was 62 µM on K562 cells. The combination of the cannabinoid type 2 receptor ligand with ponatinib in K562 cells exerted a synergistic effect (ponatinib; 0.203 µM and JWH-073; 2.70 µM), while the combined application with imatinib maintained a moderate level of synergism (İmatinib; 1.06 µM and JWH-073; 2.07 µM). The IC50 value of Ponatinib is 460 nM on K562 cells. The IC50 values of İmatinib is 3,15 µM on K562 cells. Administration of JWH-073 in combination with ponatinib significantly increased the apoptosis rate to 38.8%. As for the expression assay results, JAK2 and E2F1 genes were upregulated, while gene expressions of CNR1, CNR2, BCL-X, ABL1, MYC, TP53, STAT5A, and STAT3 were downregulated by monodose application with JWH-073 in K562 cells compared to their untreated counterparts.

Conclusion: The use of cannabinoid type 2 receptor ligand alone and in combination with the tyrosine kinase inhibitors imatinib and ponatinib showed a potential antiproliferative effect by reducing leukemic cell viability and inducing apoptosis. In addition, expression regulation in the case of the antitumor process was observed after cannabinoid type 2 receptor ligand application. Upon further investigation, cannabinoid type 2 receptor ligand could be specified as a potential combination with TKIs for the cure of CML. We think that novel cannabinoid type 2 receptors may be promising potential agents for the treatment of CML and will provide important therapeutic data.

CT P-23

Fatal intoxication by inadvertent inhalation of phosphan – a case study

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Background & Aims: A case report of a 31-year-old man, an employee of an agricultural cooperative, who was fatally poisoned by phosphane (alternate common names: Phosphine, Hydrogen phosphide, Phosphamine, Phosphorus trihydride, Phosphorated hydrogen, PH3) released during pest control of storage seeds is presented. The man was found in the residential part of the garage by the emergency services he called himself. When the emergency services arrived, the man had serious health problems, severe nausea, vomiting, and diarrhea. After a few minutes, the 31-year-old man falls into a deep unconsciousness. Resuscitation was attempted, but the subject was declared deceased after 60 minutes. During resuscitation, there is a massive flow of blood from the mouth and nose. Firefighters and police also arrived at the scene. After measuring, the firemen noted the presence of carbon monooxide in the air. The police attributed the intoxication to carbon monoxide poisoning.

Methods: Case history, autopsy, and toxicological investigation of post-mortem collected materials are described. Blood (namely blood from the lower limb), urine, liver, kidney and lung were collected post-mortem during autopsy. The biological material taken during the autopsy was subjected to a toxicological investigation with a negative result for ethanol, drug, addictive substances or carbon monoxide poisoning. After excluding the effect of ethanol, drugs, addictive substances or carbon monoxide, an investigation was carried out in the building of garages and adjacent warehouses. After an on-site investigation, a record of seed pest control treatment with phosphane was discovered. Gas chromatography flame ionization detection analysis for identification of phosphane intoxication was developed. The method was applied on analysis of collected post-mortem biological materials – blood, urine and lung. The GC-FID method was also applied for the analysis of phosphane, which was prepared in laboratory. Blood and lung samples obtained during the autopsy of the 31year-old man were put into the vial in the amount of 0.5 g of each of them. 0.1 ml of the internal standard (standard solution of methyl ethyl ketone) was subsequently

added to the vial. The vial was then closed and nitrogen was injected into the vial through the rubber cap while simultaneously exhausting the original atmosphere. Nitrogen was injected through a double needle valve (the original atmosphere was pushed out with the second needle) for 30 seconds at a constant flow rate of 20 mL min−1. Negative lung and negative blood samples were prepared in the same way. All samples prepared in this way were analyzed according to the procedure above. To determine the retention time of phosphane, an injection of a gaseous mixture of phosphane with nitrogen was used.

Results & Discussion: The presence of phosphane was proven in the biological material of the deceased. The laboratory findings of the presence of phosphane together with the autopsy findings unequivocally proved fatal phosphane intoxication.

Conclusion: In our work, a newly developed method for the determination of phosphane is described. The GC-FID method is for phosphane identification firstly reported and it is alternative possibility for identification of phosphane for clinical and forensic purposes. This method led to the clarification of the initially mysterious death of the man. On the basis of the conclusion of the autopsy and, above all, the toxicological examination, the police obtained evidence to clarify the mechanism by which the death of the young man occurred.

Effects of methamphetamine abuse on food controlling hormones (leptin and agouti related protein – AgRP) during early abstinence

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Background & Aims: Methamphetamine is a powerful, highly addictive Psycho-stimulant that affects the central nervous system. Crystal methamphetamine is a form of the drug that looks like glass fragments or shiny, bluish-white rocks. It is chemically similar to amphetamine, the drug in its pharmaceutical form used to treat attention-deficit hyperactivity disorder (ADHD) and narcolepsy, a sleep disorder. Leptin is an adipocyte-derived hormone that is secreted in correlation with total body lipid stores. Agouti is a 131 amino acid peptide made by dermal follicular cells that acts as a specific high affinity antagonist of the melanocortin receptors (MC1-R). Agouti related protein (AgRP). The hypothalamic melanocortin system plays a critical role in responding to leptin, and disruption of this system at multiple levels causes obesity in humans and animals. The aim of this study, try to understand the effect of methamphetamine abuse subjects during early abstinence on the most two popular hormones controlling food intake and energy expenditure (Leptin Hormone, and Agouti Related Protein Hormone (AgRP), in comparison with healthy subjects.

Methods: Two groups every group as the following: Group I (GI) consisting of twenty (20) men with methamphetamine abuse (as main substances) during early abstinence with BMI ranged from (18-20) kg/m2 , from Ishraq Hospital Psychiatry and addiction treatment Egypt. Group II (GII) consisting of twenty (20) control healthy males with normal weight with BMI ranged from (18-20) kg/m2, as volunteers with drug free urines. All urine samples will qualitative analysis using Gas Chromatography-Mass spectrometry (Agilent 6080N- capillary coloumn 30m-1µ diameter) collected from forensic medicin institute chemistry lab. Cairo, Egypt. Plasma leptin concentration was measured by quantitative radio immunoassay (RIA) using a kit supplied from diagnostic systems Laboratories (DSL) Inc. (445 medical center BLVB. WEBSTEER TX 77598 USA. Human plasma AgRP hormone was quantitatively measured by using a kit supplied from Quantikine R&D systems INC. (614 McKinley Palace Minneapolis, MN 55413, USA.). Age will be matched, exclusion criteria, subjects with medical illness or organic brain disorder, subjects with current psychiatric comorbidity, subjects with diabetes and hypertension, subjects under any kind of medications (psychological or organic), which can effect on appetite and food intake.

Results & Discussion: Results: There was a significant positive correlation between plasma leptin concentrations and BMI in the control groups (GII), meanwhile there was a significant negative correlation between plasma AgRP concentrations and BMI, and also between plasma AgRP concentrations and plasma leptin concentrations in the same group (GII). There was a positive correlations in (GI) between plasma leptin concentrations and BMI, but statistically insignificant. On the other hand, there was a negative correlation between plasma AgRP concentrations and BMI, and between plasma AgRP concentrations and plasma leptin, but statistically insignificant. Discussion: The interaction between methamphetamine and appetite hormones had been studied in some researches; it was found that the administration of a methamphetamine reduces plasma leptin levels in individuals. The early phase of abstinence was defined as less than 2 weeks after withdrawal, according to the previous results, methamphetamine abusers might exhibit a poor metabolic state during the early withdrawal period. We hypothesized that the decreased leptin levels might reflect the body's energy metabolic state in the abstinence period because the major physiological function of leptin is to signal states of negative energy balance and decreased energy stores. In the

hypothalamus, Ob Rbcolocalizes with NPY and Pro-opiomelanocortin(POMC) and STAT3. When leptin levels are decreased, expression of orexigenicneuropeptides agouti-related peptide (AgRP) and neuropeptide Y (NPY) is increased. In the arcuatenucleus of hypothalamus (ARH), neuropeptideY(NPY)/agouti related protein (AgRP) neurons promote feeding and are directly inhibited by leptin.

Conclusion: In the early phase of abstinence from methamphetamine abuse, there is a decrease in plasma leptin concentration. On the contrary, there is an increase in plasma AgRP concentration. This causes a negative energy balance and decreased energy stores, reflecting the state of the subjects in this phase.

CT P–25

Epidemiological, clinical, therapeutic and toxicological profile of acute intoxications: A monocentric retrospective study

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Background & Aims: Acute poisonings remain a significant cause of morbidity and mortality globally, posing a major public health challenge. In Algeria, data on the profile of acute poisonings are limited. The objective of this study, conducted at the University Hospital of Setif, was to provide an epidemiological, clinical, therapeutic, and toxicological overview of acute poisonings in this country.

Methods: The study focused on intoxicated patients admitted for acute poisoning at the emergency and medical resuscitation departments of Saadna Abdenour University Hospital in Setif.

This is a retrospective descriptive epidemiological study spanning from October 2016 to April 30, 2018. It encompassed all acute poisonings received and recorded at the University Hospital of Setif.

The data for this study was gathered from multiple sources, including the registry of emergency room consultations and medical resuscitation, as well as medical records of hospitalized intoxicated patients admitted to either the emergency room or medical resuscitation unit. Additionally, information was collected from the emergency registry of the toxicology department, which encompassed data from the information sheets provided by the requesting department, along with conducted toxicological analyses and resulting outcomes.

Results & Discussion: This study represents the inaugural investigation conducted at the University Hospital Center of Setif, focusing on analyzing the profile of acute poisonings received within the same institution.Spanning from the final quarter of 2016 to April 30, 2018, this retrospective study encompassed 406 cases.

The results showed a sex ratio of 1.41 with a female predominance. The intoxicated patients had a mean age of 24.7 ± 17.8 years, with the most affected age group being between 19 and 29 years (28%). Poisonings were mainly accidental (57%), with 18% of cases involving intentional poisonings, more common among men. Medication poisonings were the most common (54%), followed by carbon monoxide poisonings (37%).

Among the most implicated classes; psychotropic drugs come in first place, where we found antiepileptics (8.6%), followed by benzodiazepines (4.1%). Next are antipsychotics and antihypertensives (1.8%). Antidepressants are implicated in 1.3% of cases of drug poisoning. A small percentage is attributed to digoxin, morphine, and paracetamol (0.9%, 0.5%, and 0.5% respectively). Polydrug intoxication occurred in 2.7% of cases.

Carbon monoxide poisonings were exclusively accidental, while medication poisonings were intentional in 27% of cases. Cosmetics, household products, and carbon monoxide were more often implicated in cases involving females. An incidence peak statistically significant was observed in winter ($p<0.05$).

Household products, particularly bleach, ranked third (3%) in the collected acute poisonings.

The average time between poisoning and hospitalization was 15 hours and 24 minutes. Symptomatic treatment alone or combined with evacuation or detoxification treatment was used in 8.9%, 2%, and 0.2% of cases, respectively. About one-third of patients received specific management, with antidote administration only for carbon monoxide poisonings.

Conclusion: This study allowed us to assess the profile of acute poisonings at the University Hospital Center of Setif in Algeria.Their management requires emergency medical care guided by reliable toxicological knowledge. However, raising public awareness about the dangers of poisoning, reducing access to toxic products, and providing training for healthcare professionals on therapeutic management seem to be the most effective preventive tools. Awareness campaigns should be launched throughout the year to promote better storage and handling of medications, pesticides, and other chemicals involved in acute poisonings.

Socio-demographic and behavioral profile of cannabis consumers in Algeria: A retrospective monocentric study

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Background & Aims: Cannabis is a widely spread natural plant, with an increase in its consumption in recent years, especially among adolescents. According to the 2022 World Drug Report by the UNODC, cannabis remains the most consumed plant globally. In Algeria, it's an illicit drug, its production, trafficking, and abuse pose significant health and public safety problems. The dependency and toxicity effects of cannabis are well-documented, particularly its psychotoxicity, which is predominant, and risks are increased with early consumption, especially during adolescence.

The aim of this study is to profile cannabis consumers seen at the toxicology laboratory of University Hospital Center Saadna Abdennour in Sétif during 2022, to evaluate the facts of demographic characteristics, consumption habits, associations with other substances, and clinical symptoms.

Methods: This retrospective study was conducted over the year 2022 and involved 150 subjects consulting for drug-related behavior. Data were collected from information sheets including socio-professional details, frequency and habits of consumption, associations with other substances, and clinical signs. Urine samples were analyzed using an enzyme-multiplied immunoassay test (EMIT) with VIVA-E® SIEMENS. This method was used for screening benzodiazepines, barbiturates, ecstasy, cocaine, and opioids.

Results & Discussion: The study population consisted of 150 subjects, with a male predominance (90%) over females (10%). The average age was 21 ± 7years , ranging from 11 to 48 years, with a majority from urban areas (57%) compared to rural (20%). Most consultations (78.7%) originated from the Addictology Bel Air center in Sétif. Regarding cannabis consumption, 41% admitted to use, with 52.5% reporting regular and 37.7% occasional consumption, predominantly among those aged 15 to 30. In terms of family status, 90.2% were single, while 9.8% were married. Education levels varied, with a prevalence at the high school level (36.1%). Professionally, 34.4% were employed, while 62.3% were unemployed. Associations with other substances were common, with 82% consuming tobacco and 47.5% pregabalin.

Polydrug use refers to the consumption of at least two psychoactive substances. This consumption can be simultaneous, during the same occasion, or successive. Tobacco is often used in the making of cannabis joints; consequently, most cannabis users are also tobacco users.

The THC toxicology analysis showed positive results in 32.8% of subjects, and 19.7% tested positive for benzodiazepines. Approximately 20% reported clinical signs, including psychiatric symptoms such as anxiety and depression, as well as somatic symptoms like asthenia and tachycardia.

Conclusion: The high prevalence of cannabis use, particularly among adolescents, underscores the importance of awareness campaigns and training days in educating individuals about the toxic risks associated with cannabis consumption. The indispensable role of Toxicology laboratories in screening and aiding diagnosis cannot be overstated. Moreover, the collaboration between clinicians and toxicologists is essential for effectively managing cannabis dependence.

Poster gallery - DUI F-P-1 to P-17

10:00 - 10:30 Thursday, 5th September, 2024

Occurrence of semi-synthetic cannabinoids in seized cannabis products from Eastern Denmark

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Background & Aims: Cannabis has a long history of recreational use throughout the world. In recent years the cannabis landscape has been changing. Following legislation changes in many countries a new group of cannabinoids, the semi-synthetic cannabinoids, have appeared and the number of these compounds has increased rapidly in recent years. The first to be described was Δ8-THC, which can be made from a simple cyclisation of cannabidiol (CBD) under acidic conditions. Secondly, hexahydrocannabinol (HHC) has been observed, which is a reduced form of THC. Furthermore, compounds with a different number of carbon atoms in the sidechain exist, such as tetrahydrocannabiphorol (THCP). Simple derivatives of all the previous compounds could also be created, some have already been observed e.g. hexahydrocannabinol acetate (HHC-O-acetate).

In Denmark, HHC was scheduled on May 3, 2023, followed by H4-CBD, HHCP, and THCP on January 9, 2024. All isomers of THC, including Δ8-THC, have been regarded as illegal substances in Denmark since 1993.

In routine analysis of cannabis materials seized by the police and customs, only a few phytocannabinoids have been of interest. With the new developments in the cannabis market, samples are now being screened for a larger set of cannabinoids. In this study, we conducted a retrospective analysis of seized cannabis products from 2018–2023 in Eastern Denmark to investigate trends in the occurrence of semi-synthetic cannabinoids and the time of first appearance.

Methods: In Eastern Denmark, materials are seized by customs and police upon suspicion of containing illegal substances. All seized materials are screened with a gas chromatography mass-spectrometry (GC-MS) based method at the Department of Forensic Medicine, University of Copenhagen.
A retrospective data analysis of the GC-MS data from materials suspected of containing cannabinoids received for analysis in the period 2018–2023 was carried out, totalling 1441 cases.

A spectral library of semi-synthetic cannabinoids was made from available reference materials, and retention times and spectral matching were used to reanalyse the data. In addition to the GC-MS data, photos of all materials were used to divide materials into six categories: Plant material, hashish, edibles, e-cigarette products, concentrates, and dermal products.

Results & Discussion: The seized samples consisted of 587 plant materials, 220 hashish, 390 edibles, 144 e-cigarette products, 91 concentrates and 9 dermal products.

Δ8-THC was the first semi-synthetic cannabinoids to be observed in 2019 followed by HHC in late 2021. In 2022 H4-CBD and HHC-O-acetate were observed. Finally, HHCP, CBDP, and THCP were observed in 2023.

In 2023, a total of 31% of the samples contained semi-synthetic cannabinoids either in combination with phytocannabinoids or alone. For plant materials, hashish, edibles and concentrates 23-32 % of samples contained semi-synthetic cannabinoids while it was 78% for e-cigarette products and no samples contained semi-synthetic cannabinoids in the dermal products.

Conclusion: Semi-synthetic cannabinoids were initially observed in Eastern Denmark in 2019 with Δ8-THC as the first compound. Since then both the number of compounds and the number of submitted samples have increased rapidly. The presented results demonstrate the fast-changing landscape of cannabis.

DUI F-P-02

New psychoactive substances and psycoactive pharmaceuticals assessment in drivers' oral fluid samples

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Background & Aims: The Toxicology service of the Institute of Forensic Sciences of the University of Santiago de Compostela routinely performs the analysis of oral fluid (OF) samples to confirm positive results for traditional drugs (cannabis, amphetamines, opiates and/or cocaine) obtained in police roadside drug controls. Other psychoactive drugs, such as antidepressants, antipsychotics, hypnotic-sedatives or the so-called new psychoactive substances (NPS), are not currently controlled on the road; however, they can influence driving ability, putting the safety of other drivers at risk. The objective of this work was to perform a pilot study to obtained preliminary results related to the presence of these psychoactive drugs not tested in drivers drug controls by the analysis of OF samples from drivers for which a confirmation results was requested.

Methods: The OF samples (n=63) were received in our laboratory during 2023. The analysis of the traditional drugs, methadone, ketamine and some benzodiazepines is routinely performed by an accredited LC-MS/MS method using an Acquity UPLC® H-Class coupled to Xevo® TQ-XS triple quadrupole mass spectrometer (Waters Corp). For the analysis of NPS and other psychoactive drugs (antidepressants, antipsychotics and other sedative-hypnotics) an LC-QTOF-MS method was developed using an Infinity II 1290 LC coupled to 6550 iFunnel Q-TOF (Agilent Technologies), working in ESI positive mode. For chromatographic separation, an Acquity UPLC® HSS T3 (2.1 x 75 mm, 1.8 µm) column with formic acid 0.1% in water and acetonitrile as mobile phases was used. The total run time was 20 min. The acquisition was performed in data-dependent acquisition (DDA) mode (Auto-MS/MS). Data mining was carried out using Mass-Hunter Qualitative software (version B10.1) using a PCDL library built from HighResNPS [Mardal et al. J Anal Toxicol, 2019;43:520. Doi:10.1093/jat/bkz030] and our own acquired spectra. The final PCDL library includes a total of 2351 substances belonging to the most popular subgroups of NPS and the main antidepressants, antipsycotic and opia-

tes used in the clinical practice. [M+H]+ ions were recorded from 60 to 1000 m/z and fragmented using 3 different collision energies (10, 20 and 40 eV). MS/MS spectra matching was manually checked. The criteria for identification were as follows: a) chromatographic peak for the ion [M+H]+ with a intensity ≥103, b) mass accuracy (maximum error 10 ppm) and isotopic intensity and spacing for [M+H]+ providing a MS score ≥70, c) at least two product ions matching those recorded in the library with a maximum mass error of 10 ppm, and d) when the reference standard was available, a maximum retention time difference of 0.1 min.

Results & Discussion: In 4 out of 63 samples tested, none of the routinely monitored drugs or any other psychoactive drugs were detected. In the remaining 59 samples, at least one of the traditional drugs (cocaine, cannabis, amphetamines and/or opiates (heroine, morphine or codeine)) was confirmed. In 15 samples other psychoactive drug not tested in drivers' controls were also detected.

Among the traditional drugs detected, the most prevalent was tetrahydrocannabinol (THC) (69.8%), followed by cocaine (63.5%), opiates (14.3%) (morphine and/or 6-acetylmorphine, 12.7%; codeine alone, 7.9%), and amphetamine derivatives (7.9%). Polydrug consumption was frequent (50.8%).

With regard to the psychoactive drugs not detected in roadside controls, benzodiazepines or zolpidem were present in 6.4% of the samples, antidepressants in 12.7%, antipsychotics in 6.4%, ketamine in 1.6% and methadone in 4.8%. Specifically, the hypnotic-sedatives detected were diazepam and its active metabolite nordiazepam (n=2), alprazolam (n=1), lormetazepam (n=1) and zolpidem (n=1). Regarding the antidepressants, venlafaxine along with O-desmethylvenlafaxine (n=3), escitalopram (n=3), sertraline (n=1), mirtazapine (n=1), and trazodone and its metabolite m-CPP (n=1) were detected. The antipsychotic drugs detected included quetiapine (n=3) and olanzapine (n=1). In addition, the therapeutic opioids methadone (n=3), hydrocodone (n=1), O-desmethyltramadol (active metabolite of tramadol) (n=1) were also detected. None of the NPS included in the in-house library were identified in the 63 analyzed samples.

Conclusion: Results from the present study shows that combination of illegal drugs of abuse with other psychoactive drugs that can produce driving impairment is quite common, as it was observed in more than 20% of the analyzed samples. The lack of identification of these psychoactive drugs makes it difficult to know their real prevalence among drivers and their true impact on the ability to drive. Therefore, this pilot study will help to determine the magnitude of this problem.

Analyzing cannabinoids in blood and oral fluid: Implications for drug-impaired driving

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Background & Aims: In 2018, the Alabama Department of Forensic Science (ADFS) became the first state in the United States to conduct oral fluid evidentiary confirmation testing, in addition to traditional blood testing, for driving under the influence of drug (DUID) cases. The aim of this study was to evaluate the benefit of testing both blood and oral fluid specimens for the detection of cannabinoids in cases of DUID. The goal was to expand the interpretation of data support for courtroom testimony.

Methods: Over 2,000 DUID cases with blood and oral fluid specimens were evaluated from 2018-2023. Quantisal collection devices were used to collect confirmation oral fluid specimens. Biological specimens were drug screened by enzyme-linked immunosorbent assay (ELISA) using a Randox Evidence Analyzer or Tecan Evo 75 with Immunalysis reagents. Quantitation of cannabinoids was performed by liquid-liquid extraction followed by analysis using an Agilent Triple Quadrupole mass spectrometers. Cannabinoids were validated quantitatively for use in casework by ANSI/ASB Standard 036. The detection limits were: delta-9-THC (THC) at 1 ng/mL and carboxy-THC at 2.5 ng/mL (blood) and 1 ng/mL (oral fluid).

Results & Discussion: In 2023, cannabinoids emerged as the most frequently identified substance in cases of DUID, with a prevalence of 36% (590 cases), and in traffic fatalities, with a 23% prevalence (124 cases), excluding ethanol. THC had a higher positivity rate in oral fluid than blood. The mean and median oral fluid: blood ratios were 55:1 and 14:1, respectively. For oral fluid, the percentages of cases showing THC concentrations above 5, 10, 100, and 1000 ng/mL were 84%, 71%, 37%, and 5%, respectively. THC concentrations above 100 ng/mL in oral fluid suggests recent use, correlating with an increased risk of driving impairment. Conversely, in blood samples, the percentages of cases with THC concentrations above 5 and 10 ng/mL were 34% and 12%, respectively with no cases recorded above 100 or 1000 ng/mL. 44% of blood specimens had > 40 ng/mL carboxy-THC suggesting heavy or regular cannabis use. Carboxy THC was detected in approximately 5% of oral fluid specimens. The use of molar metabolite ratios and other models were explored to gain insight on recency of use.

Conclusion: The analysis of blood and oral fluid in suspected cannabis driving cases can provide important information on the potential source of impairment, recency of use, and history of use. When used in combination with other aspects of a DUID investigation, such as an officer's observations of behavior and field sobriety tests, opinions related to the likelihood of impairment can be formed to aid the trier of fact. In addition, the ability to collect oral fluid proximate to the time of driving (e.g., at the roadside) presents a distinct advantage. Laboratories should consider the use of oral fluid as an additional or alternative specimen in DUID cases, especially in jurisdictions that currently use only urine for DUID testing.

DUI P-04

Driving under the influence of psychoactive drugs in Italy: Results from roadside operative services of Italian Police Medical Officers and Forensic Toxicology Laboratory in 2016-2023

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Italian Police Force, Italian Public Safety Department, Central Health Direction, Roma, Italy

Background & Aims: Road crashes are important Public Safety problems; according to WHO they cause 1.35 million deaths and 20–50 million non-fatal injuries worldwide. Driving under the influence of drugs (DUID) increases the risk of road crashes, causing 3% of all road deaths worldwide. Cocaine, cannabinoids, opiates and amphetamine are the most frequently detected drugs in the blood of drivers involved in crashes. In Italy, fatal road accidents have steadily decreased in recent years. In 2015-2018, there were on average 173,000 road accidents/year, with approximately 3,300 fatalities. In 2019, road crashes decreased by 0.2%, with 3,173 fatalities. During the "COVID-19's lockdown", fatalities significantly decreased (2,395). However, in 2022, road crashes increased, with 3,159 deaths. In the first half of 2023, there was a slight decrease in road accidents with injuries(-1.0%) and injuries(- 0.9%) compared to 2022. WHO and UN established to half traffic injuries and fatalities within 2030 and the study of DUID'phenomenon can improve the Police Forces' prevention strategies for road safety. Italian Police Department plays a central role in the reduction of fatal road accidents, with the aim to reduce DUID-related traffic injuries and fatalities. Since 2016 specific on-road services for DUID testing have been performed, with the technical support of the Forensic Toxicology Laboratory, Medical Officers and technician health personnel of Italian Police Force. The aim of this study is to discuss the results of the Italian Police Force protocol for DUID-related fatalities' prevention and the implementation of road safety in Italy with specific prevention strategies from Italian Police Department and Government.

Methods: Over 9400 OF samples were randomly collected from drivers during specific roadside services of Italian Police Force from January 2016 to December 2023, in major Italian cities. Clinical examination and OF screening drug-tests were performed onroad by Medical Officers and technician health personnel of the Italian Police Force, using Abbott SoToxa™ Mobile Test System, Alere DDS 2 and Drager Drug Test 5000 POCs. Non-negative OF samples were collected with Quantisal Oral Fluid Collection Device (Alere) for the confirmatory forensic chemical analysis. In the Forensic Toxicology Laboratory of the Italian Police Force, OF samples were analyzed by gas cromatography coupled with mass spectrometry (Agilent Technologies 7890B gas chromatograph coupled to a 5977B mass selective detector) searching for cannabis, cocaine, opiates, amphetamine and metamphetamine. Chain of custody was guaranteed for the OF samples and the applied protocol has forensic validity. Descriptive statistical analyses were performed using anonymous data (age, sex and OF sample' collection region), combined with the psychoactive substances detected in the OF samples. The regional percentage of confirmed positive subjects was studied when controlled subjects were >50.

Results & Discussion: Due to the forced activity's stop during COVID-19 pandemic period (2020-2021), analytical data results are related to 2 periods: 2016-2019 (period 1) and 2022-2023 (period 2). In period 1, 7715 drivers were tested, 24% resulted positive at screening drugtest, and 1387 of these confirmed positive at second-level analysis. Cannabis (48.8%) and cocaine (48.2%) were the most frequently detected substances in OF samples. Users were mostly male and under 30y old. In period 2, 1756 drivers were tested, 20% resulted positive at screening drugtest and 16% confirmed positive at second-level analysis. Cannabis (59.7%) was the most frequently detected substance, followed by cocaine (38.6%). Users were mostly male and under 30y old, with a slightly lower prevalence of substance use compared to period 1. The regional distribution of DUID drivers (calculated for 17/20 regions) showed positivity rates between 5% (Friuli-Venezia-Giulia) and 26% (Marche) compared to those tested, with some exceptions. Regional differences could be related to various factors (i.e. cultural, socioeconomic, logistical). Considering drugs involved in DUID, the results on this Italian population sample seemed to agree with results from other Countries.

Conclusion: The use of psychoactive substances by drivers is a relevant issue in road safety, possible related to fatal crashes. This study reveals a wide use of cocaine and cannabis by Italian drivers, mostly male under 30 years old. The Central Health Direction of Italian Police Force with the own Forensic Toxicology Laboratory has a central

role for the implementation of specific strategies, such as the "zero-tolerance" legislation, and in achieving the ambitious goal of zero road traffic fatalities. However, it's mandatory to increase the number and the distribution of on-road DUID' checks (referring to territory, type of roads and temporal distribution throughout the week), optimizing the second-level analyses in dedicated forensic laboratories. The intensified request of analytical activity would be satisfied by the second Forensic Toxicology Laboratory of the Central Health Direction of Italian Police Force, soon opening in Northern Italy. Moreover, new roadside biological fluids' sampling methods could be tested, with a potential application of Dried Blood Spots (DBS) microsamplying. Thus, offering the possibility to study the use of New Psychoactive Substances (NPS) in DUID, enhancing specific analytical detection systems, since the EU' early warning system discovers over 50 NPS/year.

Case report of an driving under the influence case with a presumed intake of selegiline

DUI P-05

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Background & Aims: Selegiline is a monoamine oxidase-B inhibitor used for symptomatic treatment of Parkinson`s Disease. Its main metabolites are norselegiline, methamphetamine and amphetamine. To be able to differentiate between illicit use of methamphetamine and selegiline intake a differentiation between R- and S-isomers of amphetamine/methamphetamine is possible as selegiline only leads to the R-form. Analysing the samples using immunoassays will lead to lower levels or even negative results compared to the amount found in confirmatory analysis as the R-form of methamphetamine and amphetamine reacts much weaker to the immunoassay kits usually used in forensic cases. A case of a 39-year-old male person who caused a car accident with a presumed intake of selegiline is presented.

Methods: The urine sample was screened using immunoassays (Kits from Immunanalysis, Microgenics and Siemens with different techniques: CEDIA, DRI, HEIA, ARK, SEFRIA) for drugs of abuse and a LC-qTOF-MS Screening (X500R QTof and Exion LC from Sciex). All positive results were confirmed in urine and KF-EDTA-whole blood using LC-MS/MS (Shimadzu and Sciex), LC-MS-DAD (Shimadzu and Sciex) and GC-MS (Agilent). Furthermore, KF-EDTA-blood was analysed for ethanol (HS-GC-FID, Agilent). To be able to differentiate between selegiline intake and intake of illicit methamphetamine the urine sample was sent to the institute of forensic medicine of Zurich for enantiomeric separation.

Results & Discussion: Immunoassays for tramadol (HEIA, Immunanalysis) and benzodiazepines (CEDIA, Microgenics) were positive, amphetamine/ecstasy immunoassay (CEDIA, Microgenics) was 230 ug/L which is negative (cut-off 400 ug/L). In the TOF-Screening a clonazepam metabolite, lamotrigine and its metabolites, methamphetamine, ephedrine/pseudoephedrine, bisacodyl metabolites and caffeine could be detected. Neither norselegiline nor selegiline itself could be detected in urine or blood. Tramadol, benzodiazepines and amphetamines were confirmed and quantified. In urine methamphetamine with over 1000 ug/L and amphetamine with 290 ug/L could be detected by GC-MS which does not match with the immunoassay results. Furthermore, clonazepam (34 ug/L) and 7-aminoclonazepam were detected in urine. In KF-EDTA whole blood lamotrigine (2100 ug/L), clonazepam (46 ug/L), 7-aminoclonazepam (98 ug/L), tramadol (1000 ug/L), O-desmethyltramadol (210 ug/L) and methamphetamine (< 10 ug/L) could be detected. No ethanol could be detected in KF-EDTA-whole blood. Enaniomeric separation leads to R-amphetamine and R-methamphetamine which matches with the denoted intake of selegiline.

Conclusion: CEDIA amphetamine/ecstasy immunoassay (Microgenics) has a reactivity of S-amphetamine of 100%, R-amphetamine 1%, S-methamphetamine 100% and R-methamphetamine 28%. As selegiline is metabolised to R-methamphetamine and R-amphetamine the results measured with the CEDIA immunoassay of about 23% compared to the confirmatory analysis matches well with the cross-reactivity. The detected substances are consistent with an intake of selegiline, nevertheless a consumption of enantiopure R-methamphetamine instead, appearing on the illegal drug market in very low amounts, cannot be excluded as selegiline and norselegiline could not be detected in the samples.

Quantitative determination of CBD, THC and their major metabolites in low volumes of plasma samples using a fast liquid-liquid extraction LC-MS/MS workflow

DUI P-06

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Background & Aims: Cannabidiol (CBD), a major component of the Cannabis sativa plant, has demonstrated increasing therapeutic potential in clinical research for several diseases, such as epilepsy, inflammation, pain, depression, schizophrenia, Parkinson's disease, and Alzheimer's disease. Conversely, heavy repeated use of high Del-

ta-9-tetrahydrocannabinol (THC)-content cannabis has been associated with the risk of negative mental health outcomes such as psychosis, depression, and cannabis use disorder. CBD and THC are present in variable proportions either in medicinal (high in CBD and low in THC) or recreational use products (high THC and low CBD). Since millions of people use cannabis daily and its consumption for medical or recreational usage is legally permitted in a few countries, current clinical research investigates the risk factors of potential co-assumption in known diseases. This study supports such clinical challenges by providing a fast and rapid analytical method for the detection and quantification of CBD, THC, and their metabolites when only low volumes of plasma samples are available.

Methods: CBD, THC and their hydroxy- and carboxy-metabolites, 7-OH-CBD, THC-OH, and 7-COOH-CBD and THC-COOH were targeted respectively. Plasma samples were collected from clinical trials on Alzheimer's and Parkinson's diseases and only 70 µL was used for the analysis. An internal standard mixture (d3-THC, d3-THC-OH, d3-THC-COOH and d3-CBD) was added to each sample followed by 250 µL methyl-tert butyl ether (MTBE) and then vortexing for 30 seconds. All samples were centrifuged at 3000 rpm for 7 minutes. A double liquid-liquid extraction (LLE) was performed with the organic layer of each sample being removed on each occasion and the extracts subsequently combined. The extract was evaporated under nitrogen for 15 minutes at 60°C. All samples were reconstituted in 100 µL mobile phase (acetonitrile: water, 60:40 v/v) and placed in LC-MS vials for analysis via high pressure liquid chromatography–high resolution mass spectrometry (HPLC–HRMS) with a run time of 7 minutes.

Results & Discussion: This study developed and validated a targeted analytical method by using LLE-HPLC–MS/MS for the analysis of CBD, THC, and their major metabolites. The elution window for all analytes was between 3.65 to 5.20 minutes. The developed method was fully validated with an analytical range ranging from 3 to 200 ng/mL for all the analytes except 7-COOH-CBD that was 3-1000 ng/mL. Higher recovery percentages were achieved by increasing the number of extractions per sample by using MTBE as the extraction solvent. Indeed, a single step extraction provided recoveries of 50-65% for CBD, while a two-step extraction provided recoveries of >85%. The pH was not modified primarily due to the susceptibility of CBD and 11-OH-THC to isomerise to THC and THC-COOH at low pH. Plasma concentrations from clinical trials ranged from <LOQ to 80.2 ng/mL (with the highest concentration for THC-COOH).

Conclusion: The proposed sample pre-treatment procedure consisted of a fast liquid-liquid extraction with MTBE that could easily support high sample throughput. This LC-MS/MS workflow satisfies the required need of high sensitivity due to the use of triple quadrupole tandem mass spectrometry. The validated method has broader application in toxicology in cases of low volume plasma volume available and in those investigations requiring accurate quantitative measurements of the examined cannabinoids.

DUI P–07

Confirmation of cannabinoids in forensic toxicology casework by isomer-selective UPLC–MS-MS analysis in urine

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Background & Aims: Confirmation of cannabinoid use by forensic toxicology testing in urine has traditionally focused on Δ9-tetrahydrocannabinol (Δ9-THC) with analysis of its major metabolite, 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (Δ9-cTHC) in free and conjugated forms. Legalization of hemp, however, has led to widespread production and sale of cannabidiol (CBD) derivatives with psycho-activity, including Δ8-THC and Δ10-THC isomers. The increasing availability and growing use of isomer derivatives necessitates an expanded scope of cannabinoid confirmation test protocols.

We report a quantitative, isomer-selective method of cannabinoid confirmation by liquid chromatography/tandem mass spectrometry (UPLC-MS-MS) for determination of parent-drug isomers (Δ8-THC, Δ9-THC, Δ10-THC, CBD), as well as isomeric metabolites (Δ8-cTHC, Δ9-cTHC).

Methods: Optimum resolution with minimum analytical run time was achieved by employing a high efficiency solid-core particle column (CORTECS UPLC C18+, 1.6µm, 2.1 x 50 mm) eluted with a mixture of 0.1% formic acid in water and acetonitrile. Application of a step gradient, with near isocratic conditions at both the beginning and the end of the run, facilitated resolution for the early-eluting THC metabolite isomers and later-eluting CBD and THC isomers. A rapid method of hydrolysis, using the IMCSzyme ß-glucoronidase reagent, dilution, and UPLC-MS-MS analysis was employed for quantitative co-determination of free and conjugated analytes (LOD 4 ng/mL and LLOQ 10 ng/mL), using stable isotope internal standardization.

Results & Discussion: Acceptable performance was achieved for the evaluated method characteristics which included: precision, accuracy, carryover, dilution integrity, matrix effects and interference. Casework experience with the isomer-selective method revealed a 14% prevalence of Δ8-cTHC positive cases with case-to-case varia-

bility in the relative concentration of Δ8-THC and Δ9-THC metabolites. CBD prevalence was 10% and the parent drugs, Δ8-THC, Δ9-THC, and Δ10-THC, were not detected in any of the case samples. Interference studies with a prior method highlighted a limitation in the scope of traditional confirmation test protocols and a potential for interference from CBD-sourced derivatives. A comparison of Δ8-cTHC and Δ9-cTHC phase two metabolism is also reported and demonstrates parallel excretion-kinetics for the isomer metabolites.

Conclusion: The selective confirmation method for Δ8-THC, Δ9-THC, Δ10-THC, CBD, Δ8-cTHC and Δ9-cTHC in urine provides a simple and rapid method that is adaptable for routine, high-volume testing. Optimization of chromatographic separation was essential in selective analysis for the structural isomers due to similar chromatographic properties, especially the metabolite isomers. Application studies showed prevalence of Δ8-cTHC isomer in toxicology casework and reveal a significant concomitant use of Δ8-THC and Δ9-THC. Interference studies with a prior method highlighted a limitation in the scope of traditional confirmation test protocols and a potential for interference from CBD-sourced derivatives. The literature on derivative chemistry, production, and use also emphasize the need for continuing analytical vigilance in discovery of additional cannabinoid derivatives; this may require further expansion in the scope and selectivity of confirmation testing methods for psychoactive cannabinoid use.

Factors affecting the accuracy of blood alcohol determinations in clinical and forensic laboratories in Italy: A 7-year proficiency testing study

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Background & Aims: From 2017 to 2024, the Laboratory of Clinical and Forensic Toxicology (Venice, Italy) organised a national interlaboratory quality control programme for blood alcohol (ethanol) analysis by gas chromatographic methods, involving up to 50 clinical and forensic toxicology laboratories. Twice a year, two whole blood samples containing known amounts of ethanol were prepared and sent to all participants for analysis as a proficiency test. The sample preparation procedure was validated by assessing the homogeneity and stability of the prepared samples according to ISO 13528:2015 and ISO 17043:2010. In particular, different transport and storage conditions were tested to ensure adequate temperature control according to the expected time of sample delivery (within 24 hours for internal destinations and 48 hours for insular areas).

Methods: For each round, participants were asked to complete an online form providing details of their laboratory methods and analytical procedure for ethanol determination. In particular, the following information was collected together with the analytical results: the analytical technique used, the type of storage conditions, the time elapsed between sample delivery and analysis, the reference material used, the number of replicates performed, the sample volume taken for each analysis, the measurement uncertainty associated with the analytical results provided, and whether the laboratory was accredited or not. The distributions of the z-scores (n=1171) were analysed for these variables and the main factors influencing the accuracy of the results were identified.

Results & Discussion: The use of matrix-matched certified reference materials, for calibration and/or quality control, was found to be the most important factor influencing results, with the largest positive effect. The combined effects of matrix-matching and certification result not only in average z-scores closer to 0, but also in narrower distributions of reported results, both of which contribute to accuracy. Other factors (e.g. number of replicates, volume of sample taken for analysis) played a less important role. The accreditation of the laboratory and the method of ethanol analysis were not found to influence the reliability of reported results. The potential confounding effect of the reference material on other variables was assessed and taken into account in the processing of the results. The study provides a summary of the results and a specific discussion for each factor investigated.

Conclusion: Participation in the proficiency testing programme improved the overall performance of the Italian laboratories over time. The accuracy of blood alcohol determinations by chromatographic methods was mainly influenced by the type of reference material used for internal quality control purposes. In particular, the combined effects of matrix-matching and certification were the most important factors with the largest positive effect on z-scores.Participation in the proficiency testing programme improved the overall performance of the Italian laboratories over time. The accuracy of blood alcohol determinations by chromatographic methods was mainly influenced by the type of reference material used for internal quality control purposes. In particular, the combined effects of matrix-matching and certification were the most important factors with the largest positive effect on z-scores.

DUI P-09

Consideration on the determination of blood alcohol concentration (BAC) using Widmark formula in Korea

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Background & Aims: Widmark formula is commonly used for the determination of a blood alcohol concentration (BAC) with factors such as the consumed alcohol amount, weight and a gender constant (rho factor). It has been used for forensic purpose against driving under the influence (DUI) in Korea since 1986. Although, many studies have been conducted to make the BAC calculation more accurate with respect to the factor, we have not applied them to forensic field. Therefore, there is need to update the calculation details and establish a guideline which can be used in both forensic institute and the courts.

Methods: We conducted drinking experiment on 44 healthy Koreans(11 female and 33 male) assigned to drink 1 g of ethanol per kg body weight within an hour and BAC curves were determined. And also we referred other published data and literature.

Results & Discussion: Experimental condition was somewhat harsh and not realistic: early morning drinking, with little food after overnight fast, a rather large amount of alcohol. So estimated initial BACs from C-T curves were not much different with Widmark's calculations. Consumed alcohol cannot fully reaches the systemic blood though. So considering bioavailability (F) is suitable in social case. The linear time interval and elimination rate (β) in BAC curves was similar to other BAC measurement cases.

For the determination of a BAC using Widmark formula, estimation of total body water (TBW) using anthropometric equation is appropriate for individuals rather than the static gender factor. And Watson equations validated in majority of races including Koreans can be preferred.

At low BAC, Km and Vmax of hepatic class I ADH were used to set the LambertW function.

Conclusion: We are planning to recommend the following guideline to the police and the courts in Korea.
1. To apply bioavailability (F) range of 70-90 % to Widmark formula except for the amount of alcohol in post-incident drinking.
2. To use Watson equations for total body water (TBW) estimation and then calculate an individual's volume of distribution (Vd) instead of a gender rho factor.
3. A range of elimination rate (β) is 0.010~0.025 g/dL/h (mean 0.019 g/dL/h).
4. Retrograde extrapolation using β can be performed between 1.5 hour after last drink and the time when BAC is 0.020 g/dL.
5. To use LambertW function derived from Michaelis-Menten equation at BAC below 0.020 g/dL.

DUI P-10

The study on stability of ethanol and metabolite in stored human blood samples

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Background & Aims: In medicolegal investigations, ethanol detection presents significant challenges, notably the delays between the discovery of a body and the actual time of an incident, as well as issues related to the long-term storage and transportation of bodies or samples. Microbial fermentation may induce ethanol production in biological specimens that were initially ethanol-free, complicating postmortem analyses. Additionally, the risk of external ethanol contamination is a significant concern. Ethyl glucuronide (EtG) and ethyl sulfate (EtS) serve as biomarkers for detecting ethanol consumption. Yet, it is uncertain whether ethanol produced in blood from decay or external contamination contributes to the formation of EtG or EtS. Concerns about microbial fermentation causing false positives for these metabolites have been noted in the literature. Notably, some studies have detected EtG in human blood samples spiked with ethanol and stored at 37°C for 24 hours. Our study aims to evaluate the reliability of EtG and EtS in identifying the sources of ethanol in blood under varied conditions.

Methods: Twenty blood samples, all initially negative for ethanol, n-propanol, EtG, and EtS, comprised fourteen post-mortem (PM) blood samples and six fresh venous blood samples. These were divided into two groups at 37°C, ethanol-spiked group (n=20) and control group (n=20). The ethanol-spiked group was treated with ethanol to achieve a final concentration of 1.6 mg/mL. This concentration is double the legal blood alcohol concentration (BAC) limit for impaired driving and is close to the average BAC (1.51 mg/mL) found in alcohol-positive drivers who died in traffic crashes in Shanghai, China, according to a previous study. The control group was treated with an equivalent volume of sterile, enzyme-free saline. We monitored the concentrations of ethanol, n-propanol, EtG, EtS at intervals of 0, 1, 5, 7, 9, 11, 13, 15, 17, 20, and 23 days.

Ethanol and n-propanol levels were analyzed according to the national standard (GA/T 842-2019). EtG and EtS were extracted using the Solid Phase Extraction method: we activated the cartridges with 1.2 mL of methanol (MeOH) containing 0.3% formic acid followed by water; loaded the samples and then washed them with 1.2 mL of methanol containing 0.3% formic acid. We eluted the samples with 1.2 mL of methanol containing 5% ammonia water ($NH_3 \cdot H_2O$), evaporated the eluent, and redissolved the residue for analysis. The limit of detection of ethanol, EtG and EtS were 10μg/mL, 10ng/mL and 20ng/mL, respectively.

Results & Discussion: Stability of ethanol
The ethanol concentration in the ethanol-spiked group showed a trend of first increasing and then decreasing, and reached the peak on the fifth day. However, the ethanol concentration in fresh venous blood samples decreases faster than in PM blood samples. The ethanol concentration of PM blood samples in the control group showed a same trend with fresh venous blood samples, whereas the concentrations were different. The maximum concentrations of ethanol produced in PM blood samples and fresh venous blood samples were 0.644mg/mL and 0.015 mg/mL, respectively. N-propanol was detected in only one of the PM blood samples of control group, and the ratio of ethanol to n-propanol was less than 20.

Stability of EtG and EtS
EtG was present in three of twenty samples spiked with ethanol after 9 days, the contents were 31.2, 15.0 and 10.0 ng/mL, respectively. No EtS was found in any of the ethanol-spiked samples. The presence of EtG in some samples and the absence of EtS highlight the variable formation and stability of these metabolites under identical conditions, suggesting that other factors such as microbial flora might influence metabolite formation.

Conclusion: Forensic experts should avoid relying solely on EtG to identify the source of ethanol in postmortem blood, especially if the body was discovered in high temperatures (like 37°C) or if only a small amount is detected, particularly in the absence of additional corroborating evidence. Utilizing a singular biomarker to assess antemortem alcohol consumption carries the potential for false positive outcomes.This study was supported by National Key Research and Development Program of China (No. 2017YFC0803504 to Keming Yun) and Shanxi Province Key Research and Development Program (No. 202302130501007 to Keming Yun)

Evaluation of harmful drinking among professional drivers by direct ethanol biomarkers and its relation with psychological distress

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DUI P-11

Background & Aims: Professional drivers face elevated stress due to irregular schedules, prolonged hours, and challenging conditions, leading to heightened susceptibility to stress, anxiety, and depression. In Brazil, land transport, mainly by trucks, constitutes about 80% of the nation's transportation, with an estimated two million truck drivers, mostly male. Despite breath alcohol tests, specific biomarker-based tests for chronic alcohol use aren't required for Brazilian drivers.

Currently, alcohol consumption assessment relies on self-reported questionnaires like AUDIT and AUDIT-C, which may be biased. Recent research involving direct ethanol biomarkers has shed light on substantial underreporting in self-reported questionnaires. Characterized by a half-life of approximately 4 to 7 days, with a detection window spanning one month, phosphatidylethanol (Peth) stands as a tool for monitoring abstinence and distinguishing between safe and hazardous drinking patterns. In addition, ethyl glucuronide (EtG) and ethyl sulfate (EtS) are biomarkers of recent alcohol intake in blood, with a shorter detection window. This study aims to assess alcohol consumption among drivers and its correlation with anxiety, depression, and stress. It involves measuring Peth, EtG, and EtS in dried blood spots (DBS) samples alongside self-reported AUDIT-C data. This represents an initial exploration of harmful drinking using direct ethanol biomarkers while examining psychological distress among professional drivers.

Methods: The study was carried out in South Brazil. Professional drivers possessing licenses for operating buses or trucks were recruited. Data collection took place during regular working hours from Wednesday to Friday. Prior to participation, all individuals signed the Informed Consent Form. participants signed the Informed Consent Form. Ethical consent for this study was granted by the research ethics committee of Feevale University (Approval Number: 5.082.335). The assessment of potential harmful drinking was conducted through the measurement of direct biomarkers: phosphatidylethanol (Peth), ethyl glucuronide (EtG), and ethyl sulfate (EtS), using dried blood spots (DBS). Additionally, self-reported data from the Alcohol Use Disorders Identification Test (AUDIT-C) were used. Emotional states, including depression, anxiety, and stress, were evaluated using the Depression, Anxiety, and Stress Scale (DASS-21).

Results & Discussion: A total of 97 participants were involved in the investigation, predominantly comprising male individuals (96%) and identifying as truck drivers (75.3%). Among this cohort, 43.3% acknowledged engaging in work exceeding 10 hours per day. The majority of respondents demonstrated normal levels of stress (81.4%), anxiety (83%), and depression (86.6%). Utilizing the AUDIT-C assessment tool, 30.9% were categorized as exhibiting moderate risk behaviors related to alcohol consumption, while 11.3% were identified as having a high risk. Ethyl glucuronide (EtG) and ethyl sulfate (EtS) levels, indicative of recent ethanol intake, were detectable in 14.4% of the drivers. Conversely, the longer-lasting metabolite Peth (16:0-18:1) was present in 88.7% of the participants. A moderate correlation (rs= 0.45, $p < 0.01$) was observed between Peth levels and AUDIT-C scores. Employing a Peth threshold of ≥ 59.0 ng ml-1, the Receiver Operating Characteristic (ROC) curve demonstrated 78% sensitivity and 73% specificity in accurately discerning high-risk alcohol consumption patterns. Importantly, no significant associations were discerned between alcohol consumption and levels of stress, depression, and anxiety.

Conclusion: The research outcomes reveal a significant portion of drivers partaking in consistent alcohol consumption alongside demanding work schedules. Particularly noteworthy are the findings from Peth measurements, which underscore discrepancies between self-reported AUDIT-C data and actual alcohol consumption levels. These findings provide compelling evidence for the incorporation of biomarkers in evaluating alcohol consumption behaviors among drivers.

DUI P-12

Blood drug analysis and substance use profile in cases suspected of driving under the influence of drugs in Kayseri, Turkiye

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Background & Aims: There is extensive research in the literature that substance use increases traffic accidents by impairing the visual, cognitive and/or motor abilities necessary for safe driving. This poses a threat to public safety for other drivers and pedestrians.

In cases of driving under the influence of drugs, blood substance analysis is extremely valuable because it shows being "under the influence of drugs" and is closed to adulteration. Therefore, it has important evidentiary value in forensic toxicology.

In this study, we aimed to investigate the rates of substance positivity and the most detected substances in blood samples admitted to our laboratory with suspicion of driving under the influence of drug within the past year, and to reveal the current status of substance abuse in our city.

Methods: Between March 1, 2023 and February 15, 2024, blood samples (n=327) admitted to Erciyes University Drug Application and Research Center Medical Biochemistry Laboratory by law enforcements for drug screening and confirmation analyses were included in the study. Following liquid-liquid extraction, precipitation and evaporation pretreatments, the blood samples were analyzed using a validated method on a SCIEX Triple Quad 5500+ QTRAP liquid chromatography-mass spectrometer (LC-MS/MS). A total of 90 substances were screened and the total run time was 10 minutes. Results were quantified by analyte/IS areas on Sciex OS® software and spectral validation was performed using the mzCloud Mass Spectral Library. For positive samples, demographic data, detected substances and their blood levels were reviewed

Results & Discussion: During the specified period, a total of 327 blood samples were admitted to the laboratory for substance analysis, belonging to individuals aged between 12 and 77 years (mean age 32.8 years), including 24 (7.3%) females and 303 (92.7%) males. Of these, 79% (n=259) tested positive for at least one substance above the limit of quantification (LOQ), with single substance abuse detected in 62% (n=204) and multiple substance detected in 17% (n=55). Indeed, 21% (n=68) tested negative.

The mean age of the positive group was 32.6 years (range 16-58) and the mean age of the negative group was 33.5 years (range 12-77) (p=0.473). While 95.4% (n=247) of the positive group was male, 82.4% (n=56) of the negative group was male (p=0.001).

Methamphetamine and amphetamines were the predominant substance group detected in blood (86.1%), followed by cannabis (13.9%), while cocaine was the least frequently detected substance (1.2%). Additionally, 13.9% (n=36) of samples tested positive for substances beyond the standard five-panel screen (amphetamines, opiates, cannabis, cocaine, and benzodiazepines), with pregabalin (52.8%) and gabapentin (36.1%) being the most frequently detected substances within this category.

According to the cut-off values determined in the "Driving under the Influence of Drugs, Alcohol, and Medicines (DRIUD)" project, the rate of positive samples above the cut-off value were considered as individuals under sub-

stance influence and the rate was 88.8% (n=198) for the amphetamine group, 61.1% (n=22) for the cannabis group, 44.4% (n=4) for the benzodiazepine group, 16.6% (n=3) for the opioid group, and 66.6% (n=2) for the cocaine group, resulting in an overall rate of 79.2% (n=229).

Our study presents the results of blood drug analysis and the substance use profile of cases associated with suspicion of driving under the influence of drugs. At least one substance above the quantitation limit was detected in 79% of suspected cases. In addition, polysubstance abuse was detected in almost one in five cases (17%). The most commonly abused substances were amphetamines and cannabis. Furthermore, the detection of substances beyond the standard five-panel screen in a significant proportion of cases (13.9%) indicates that pregabalin and gabapentin use is widespread in Turkey and suggests that screening analyses should be more comprehensive to include these substances.

Substance concentrations above the cut-off value indicating that drivers were under the influence of drugs were detected in more than half of the cases. These findings confirm the association of substance use with driving impairment and demonstrate once again that it is a widespread problem in society.

Conclusion: The present findings of our study emphasize that methamphetamine and amphetamines are the mostly abused substances for drivers and pregabalin and gabapentin are the most accompanying drugs in Kayseri city in Turkiye, which may pose a serious threat to traffic safety problem.

Cocaine in traffic accidents : A Study of three cases

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Background & Aims: While the causal link between road accidents and alcohol consumption is well established, numerous studies and observations have also shown that cannabis, cocaine, amphetamines, and opioids are often involved in such incidents. In Algeria, over the past four decades, combating driver alcohol consumption has been a priority for public authorities to improve road safety. Law No. 01-14 of August 19, 2001, amended and supplemented by Law No. 04-16 of November 10, 2004, and Ordinance No. 09-03 of July 22, 2009, sets the legal blood alcohol levels in its Article 2 and outlines the penalties for driving under the influence of alcohol in its road traffic code in Articles 66, 67, and 68. Article 19 states that driving under the influence of narcotics is prohibited, without further details.

Methods: In this study, we report three cases of cocaine consumption that arrived at the toxicology service for screening in a medical emergency context following a road accident during the year 2023.

Results & Discussion: Results: The first case (C1) involves a 28-year-old woman brought to the emergency department by the police in a state of agitation, with no prior medical history. The second case (C2) is a 21-year-old man brought in due to altered consciousness and agitation. The third case (C3) also involves a 21-year-old man who exhibited euphoria upon admission, a loss of contact with reality, and bilateral mydriasis. Laboratory toxicology analysis of urine and blood samples confirmed the presence of cocaine in all three cases. Concentrations were higher than 300 ng/ml in urine and above 50 ng/ml in blood for benzoylecgonine (BZE), and above 200ng/ml for cocaine in urine. Tests were negative for alcohol and other drugs and psychotropic substances, including cannabis, opioids, ecstasy, barbiturates, tricyclic antidepressants, antipsychotics, and benzodiazepines, except for C2, where the toxicological analysis revealed the presence of benzodiazepines and tricyclic antidepressants in addition to cocaine.

Discussion: The results of our toxicological analyses indicate that all three drivers were under the influence of cocaine at the time of the accident. However, for driver C2, the analysis also revealed the presence of benzodiazepines and antidepressants. This suggests that acute cocaine use could play a direct role in the occurrence of road accidents. Indeed, cocaine can lead to aggressive driving as well as errors in attention or judgment, increasing the risk of losing control of the vehicle.

Conclusion: This study highlights the involvement of illicit substance consumption, particularly cocaine, in road accidents in Algeria. Our observations of three distinct cases of cocaine overdose leading to medical emergencies following road accidents underline the danger of driving under the influence of such substances. These cases demonstrate significant behavioral and perceptual alterations, leading to increased risks on the road. Although Algerian legislation is strict regarding driving under the influence of alcohol, it must continue to evolve to more effectively address the growing problem of driving under the influence of drugs. It becomes imperative to enhance awareness, screening, and sanctions regarding the use of narcotics while driving, thereby aligning the perception of risk of these substances with that of alcohol

DUI P–14

Psilocybin, microdosing and impairment risk

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Background & Aims: Psilocybin, an indolealkylamine, is the primary psychoactive compound of "psychedelic" mushrooms. In the U.S., psilocybin is controlled as Schedule I; however, allowances are being made to enable psychedelic-assisted therapy and decriminalized personal possession.

Micro-dosing psilocybin has recently gained popularity, where users ingest a "sub-hallucinogenic" or "sub-perceptual" dose, usually with the intention to increase mood or creativity. The ramifications related to driving impairment and/or other safety-sensitive functions are of concern. This study reviews existing literature on psylocibin micro-dosing practices to identify information gaps in our ability to evaluate impairment risks.

Methods: A literature search was conducted to identify relevant peer-reviewed articles. The search terms "micro-dosing", "micro dosing", "microdosing" and "psilocybin" were employed, and 74 articles were identified and ultimately 59 of the articles were relevant to this work (n=59). Each article was assessed for targeted information related to impairment and risk.

Results & Discussion: Five types of articles were identified: case reports (8.5%), animal studies (11.9%), clinical studies (15.3%), qualitative data (27.1%), and review articles (30.5%). Four articles (6.8%) did not fall into these subcategories and were focused on theoretical information such as the mechanism of action, dose, and perspectives on microdosing. Fifteen (25%) articles noted impairment, and of these, 13% were animal studies, 33% clinical studies, 27% qualitative data, and 13% review articles. When noted, impairment was characterized based on self-reported measures or subject observation (animal and human). Cognitive impairment was the most often reported impairing effect noted (~47%, n=15), with variability in how it was described. Anxiety, nervousness, and physical tension were the second most often reported impairing effects (~38%, n15). Safety sensitive functions related to impairment such as detriments to locomotion, vision, and environmental focus were noted.

Conclusion: Downstream effects, such as impaired driving, were not addressed in any of the 59 articles related to mirco-dosing of psilocybin. There were no articles that included assessment of impairment or impairing side effects as the primary focus or as an intended component of the research. Although these studies highlight therapeutic potential of micro-dosing, work is needed to address the potential risk to patient and public safety associated with this practice.

DUI P–15

The effect of dietary supplementation on the rate of elimination of ethanol in breath

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Background & Aims: Breath alcohol concentrations (BrAC) often require extrapolation to a likely alcohol level at the time of an incident where certain caveats, and "average" expected rates, in conjunction with case circumstances, are considered when forming an interpretation in medicolegal investigations. Although alcoholics develop faster ethanol elimination rates as a result of metabolic tolerance and enzyme induction, and many drug-drug interactions have been explored in forensic toxicology, the effect of dietary supplements on the elimination of ethanol remains underexplored. With many supplements on the market claiming to increase metabolic rate and being promoted as "hangover cures", it is particularly important to ascertain whether extrapolated BrACs based on expected rates of elimination yield underestimated concentrations at the time of an incident.

Methods: Ethical approval was sought from the University of Greenwich Faculty of Engineering and Science for a pilot study requiring a prospective and longitudinal design involving ethanol consumption. Seven volunteers were asked to consume ethanol (between 2-4 units, or 50-100mL 40 % ABV). Their breathalyser readings were measured every 15 minutes on a Draeger Alcotest 7510 breathalyser until the readings measured 0.00 ug/100mL breath. The volunteers then commenced a regime of at least 2 weeks' of apple cider vinegar supplements (Apple Cider Vinegar, Holland and Barrett's Apple Cider Vinegar tablets, or Holland and Barrett's Apple Cider Vinegar gummies), Turmeric capsules (Holland and Barrett) or "Active Liver" (New Nordic supplements containing milk thistle, artichoke, turmeric and choline). Subsequently, volunteers consumed the same dose of ethanol under the same conditions and the rates of ethanol decrease were compared with the rates prior to the supplementation regime, with every volunteer acting as their own control. All breathalyser readings were measured in duplicate from which average values were calculated.

Results & Discussion: Following consumption of ethanol, the rate of elimination was calculated in the post-absorptive phase of the BrAC-time profile. While the elimination rate in breath prior to supplementation consumption was 0.087 (± 0.034) mg/L/hr, the rates differed following supplementation. For example, after the consumption of apple cider vinegar supplements, the post-absorption rates decreased unexpectedly to 0.072 (± 0.019) mg/L/hr indicating a slower rate of metabolism based on BrAC readings. The biggest decrease in rate involved a female who had consumed apple

cider vinegar gummies. As the investigations are still ongoing, the metabolic changes following the consumption of the other supplements will also be discussed. Further studies involving multiple repeats on the same individuals and supplementation for a longer period of time are necessary to see whether there could be a more pronounced effect. Studies need to be conducted to determine whether there is a possibility of faster metabolism induced by other supplements and understand the mechanism of supplement-alcohol interactions. This is needed to determine the possible deviation of supplement-induced metabolic rates from typical metabolism rates currently used as the forensic range.

Conclusion: More investigations into alcohol-supplement interactions are needed as although claims regarding supplements are typically anecdotal, they have crucial implications to medicolegal investigations. The use of expected rates of metabolism (slope/gradient) to extrapolate back to the BrAC at the time of an incident (and therefore the associated blood concentration) may yield an underestimate or overestimate which in turn has the potential to lead to wrongful conclusions about the likely alcohol impairment at the time of driving.

Evaluating the occurrence of delta-8-THC in whole blood samples taken from drivers arrested under Section 4 and Section 5 of the UK Road Traffic Act

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Background & Aims: This work describes the occurrence of delta-8-tetrahydrocannabinol (d8-THC) in whole blood in drug driving related cases, when there is a mixture of d8-THC and delta-9-tetrahydrocannabinol (d9-THC) present.

d8-THC is a naturally occurring cannabinoid and a positional isomer of d9-THC, which differs by the location of the double bond in the alicyclic ring. It is found within hemp and marijuana plants, however the naturally occurring source of d8-THC isolated from cannabis is typically <1%1. The psychoactive properties of d8-THC are qualitatively similar to d9-THC, although the d8-isomer shows lower potency ranging from 50% to 75%2,3.

Since March 2015, d9-THC has been one of the 17 compounds covered under Section 5A of the UK Road Traffic Act. This states that it is illegal to drive or attempt to drive with a specified controlled drug in blood above a specified limit. d8-THC is not covered under this Act. Both the Forensic Toxicology and Forensic Drugs departments at Eurofins Forensics Services (EFS) have seen a gradual increase in the number of samples positive for d8-THC since starting to monitor for its presence in 2022. These samples are representative of a data set that consists of submissions provided by police forces from across the UK. From anecdotal evidence, the increase in the appearance of d8-THC is believed to be due to the popularity of extraction of cannabidiol (CBD) from hemp plants and the subsequent conversion to d8-THC. As CBD is extracted from herbal material and then converted to d8-THC, the Forensic Drugs department were observing d8-THC in cannabis extracts (oils, edibles etc.) rather than in herbal cannabis.

Methods: Over 11,000 whole blood samples, from a time period between August 2023 and February 2024, were examined for the presence of d8-THC and/or carboxy-d8-THC. A triple quadrupole (Shimadzu) LC-MS/MS in MRM mode was used to screen for the d8-THC and carboxy-d8-THC. Confirmation of the d8-THC and carboxy-d8-THC was undertaken by a triple quadrupole (Agilent) GC-MS/MS in MRM mode. Semi-quantification of d8-THC was achieved on the GC-MS/MS method using the m/z 343 fragment ion from the d8-THC measured against the d9-THC calibration curve.

Results & Discussion: Over the time period, 214 cases (~2% of cases analysed) were identified with d8-THC and/or carboxy-d-8-THC present. Concentrations for d9-THC ranged from 0.1 to 14.2 ng/mL and approximately 0.15 to 230 ng/mL for d8-THC. In a small number of cases an interfering compound caused ion ratios to fail the acceptance criteria for d9-THC. The peaks for d8 and d9 compounds were sufficiently separated so d8-THC was not the source of interference and further investigation work is ongoing. The number of cases in which d8-THC and carboxy-d8-THC was detected increased each month over the seven-month period.

Conclusion: The occurrence of d8-THC and carboxy-d8-THC detected in samples is on the increase in cases that are representative of the population of samples taken for driving under the influence of drugs arrests in the UK. In some (n=8) cases, d8-THC was detected at an estimated concentration that was greater than the specified limit for d9-THC. In future, this may present a challenge to the current S.5A Road Traffic Act law.

1. Jami D Reber, Erin L Karschner, Joshua Z Seither, Jessica L Knittel, Katherine V Dozier, Jeffrey P Walterscheid (2022) An Enhanced LC–MS-MS Technique for Distinguishing Δ8- and Δ9-Tetrahydrocannabinol Isomers in Blood and Urine Specimens, Journal of Analytical Toxicology, Volume 46, Issue 4, Pages 343–349, https://doi.org/10.1093/jat/bkac007

2. Karniol I.G., Carlini E.A. (1973) Comparative studies in man and in laboratory animals on Δ8- and Δ9-trans-tetrahydrocannabinol. Pharmacology, 9, 115–126.

3. Hollister L.E., Gillespie H.K. (1973) D8- and d9-tetrahydrocannabinol comparison in man by oral and intravenous administration. Clinical Pharmacology & Therapeutics, 14, 353–357.

DUI P-17

Evaluation of BAC levels determined in traffic accident cases admitted to the emergency department in Adana, Türkiye

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Background & Aims: Around 1.35 million people die in traffic accidents year, 20 to 50 million get non-fatal injuries, and many more become disabled as a result of their injuries, according to numbers from the World Health Organisation (WHO) for 2020. Previous studies have found a link between driving under the influence of alcohol (DUIA) and an increased risk of mortality and injury in traffic accidents. According to Turkish laws and regulations, the legal alcohol limit for drivers is 50 mg/dL. In addition, drivers found with a BAC of more than 100 mg/dL are subject to additional penalties for endangering traffic. The aim of this study was to investigate the blood alcohol concentration (BAC) levels and demographic features of people who were sent to the emergency laboratories of university hospitals after being involved in traffic accidents, occupational accidents, and forensic cases in Adana in 2021.

Methods: Patients who were evaluated for BAC levels in the emergency department were included in this study. Blood samples of the 1266 cases were analyzed with GC-Headspace method in 2021. The data were obtained from the hospital's automation system and were statistically analyzed.

Results & Discussion: Of all cases (n = 1266) admitted to the hospital emergency department, the rate of traffic accidents was 74.0 % (n = 937), forensic cases was 23.1% (n = 292), occupational accidents was 1.3 % (n = 16) and emergency cases was 1.7 % (n = 21). The majority of the traffic accident cases consisted of males, and 2.9 % (n = 27) of these cases were under 18, which is the legal age for receiving a license permit. The mean age was 35 ± 14.6, and 71.9 % of the cases were male. Ethyl alcohol was positive in 10.0 % (n = 93) of the cases and 39.8 % (n = 37) of them were in the 25–34 age range. About 84.0 % (n = 78) of the positive cases were above the legal limit of 50 mg/dL.

Conclusion: Driving under influence of alcohol is a significant risk factor in our country as it is in the world. This study presented the detection of ethyl alcohol in traffic accidents in the Adana region to provide an overall picture of Türkiye. Continuous and systematic future studies can help monitor the problem of alcohol use.

Poster gallery - GT P-1 to P-29

10:00 - 10:30 Thursday, 5th September, 2024

GT P-01

Hazardous weed jellies and capabilities of field drug detection techniques

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Background & Aims: Cannabis, known locally as "bhang," has a long history of traditional use in Pakistan, particularly in certain regions such as the northwestern frontier provinces. It's often consumed in the form of a drink made from the leaves and flowers of the cannabis plant.

In recent years, there have been reports of increased interest in cannabis-based products globally, including edibles like gummies and candies infused with cannabis extracts. It's possible that similar products might be emerging in various markets, including Pakistan, despite the legal restrictions.

It's important to note that the sale, possession, and use of cannabis or any cannabis-derived products in Pakistan are illegal under the "Control of Narcotics Substance Act 1997", and can lead to legal consequences. Additionally, the quality and safety of illicit cannabis products can't be guaranteed, potentially posing health risks to consumers

Allegedly, certain students were discovered using a substance known as "Ice Jelly" to induce intoxication, although they lacked understanding regarding its composition. They obtained this product via courier from an unidentified individual, solely possessing a WhatsApp contact number for communication. Upon investigation, the students claimed ignorance regarding the contents of the substance. A sample of the product was collected from them and subsequently transferred to the authors' laboratory for forensic analysis aiming the detection of active drugs in the product and the reason behind its non-detection in field if any.

Methods: For the analysis, Raman-based handheld analyzers, including the Thermofisher True Narc and Rigaku Progeny Res Q, were employed to assess the field detection capabilities. The Thermofisher True Narc is equipped

with a laser excitation source, operating at a wavelength of 785 nm, and offers a spectral range from 200 to 2000 cm^-1. Similarly, the Rigaku Progeny Res Q utilizes a 1064 nm laser excitation source and provides a spectral range from 176 to 3314 cm^-1. These devices were utilized under ambient conditions.

Chemical spot testing using a Modified Duquenois Levine assay was conducted as a complementary method. Subsequently, the substance under investigation was dissolved in methanol, filtered, and subjected to Gas Chromatography-Mass Spectrometry (GCMS) analysis using an SDP TOP GC1290 coupled with an MS8100 detector. The GCMS analysis was conducted according to a previously developed and validated method for cannabinoid detection in the laboratory.

Retention times obtained from the GCMS analysis were compared with reference standards for cannabinoid compounds. Moreover, the mass spectra generated were automatically matched with databases including the NIST library and the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) version 3.13 database for further confirmation and identification of compounds present in the sample.

Results & Discussion: During the analysis, the Thermofisher True Narc consistently provided inclusive results in each attempt. Conversely, the Rigaku Progeny Res Q demonstrated variability across different analyses. Initially, it detected a mixture comprising starch protein and DIPT (N,N-Diisopropyltryptamine). Subsequently, it identified another mixture containing phosphorous chloride and D-penicillamine. Lastly, the Rigaku Progeny Res Q indicated the presence of a mixture consisting of cholesterol steroid and starch protein.

The Modified Duquenois Levine assay exhibited a perfect purple over pink layer, indicating a positive result.

GCMS analysis revealed three distinct peaks corresponding to cannabidiol, delta 9 tetrahydrocannabinol, and cannabinol at retention times of 13.69, 14.13, and 14.41 minutes, respectively. The mass spectra also exhibited a match quality exceeding 90% for all the cannabinoids, providing high confidence in their identification. Additionally, benzyl alcohol and phloroglucinol were detected among the other considerable peaks during GCMS analysis

Conclusion: The use of cannabis products, particularly in edible forms, poses various hazards and potential consequences. Legal status of cannabis varies worldwide, with some countries legalizing its medicinal and/or recreational use, while others maintain strict prohibition. In Pakistan, cannabis and its derivatives are illegal under the Narcotics Control Act, and possession or use can result in legal consequences.

Detection of drugs in complex mixtures presents challenges, especially in field drug detection where handheld analyzers employing Raman analysis may have limitations in identifying substances without any separation technique. Color tests can be more efficient in this regard, targeting the specific analytes.

Our study, the first to report such cannabis products in Pakistan, also indicates the presence of solvent residues and added prescription drugs, such as Phloroglucinol. Phloroglucinon might have been added for certain effects not fully investigated. Long-term abuse of such materials can pose serious health threats, including toxic effects from residual solvents and potential adverse reactions from undisclosed additives.

Such products carries many unknown health as well as legal consequences. Comprehensive analysis and regulatory measures are essential especially in youngsters to address these risks and protect public.

Significance of gastric contents for detection of para-phenylenediamine (PPD) and its metabolites

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Background & Aims: Para-phenylenediamine (PPD) is a deadly poison known as 'Black Stone' present as an ingredient in temporary tattoos and hair dyes. Ease of accessibility, cheap cost, and ease of preparation by dissolving it in water or any other beverage make it a preferred poison for suicides and homicides. Oral poisoning in high doses causes angioedema and fatal arrythmias in the initial 6-24 h while moderate doses cause acute renal failure due to rhabdomyolysis resulting in death within a week. This study signifies the sample of choice (antemortem and postmortem) in comparison to other biological specimens for the qualitative detection of PPD and its metabolites (monoacetyl p-phenylenediamine and diacetyl p-phenylenediamine).

Methods: Blood, urine, gastric contents, and liver were screened for the presence of PPD using a colorimetric principle-based assay. Blood, urine, and gastric contents had no pre-treatment, while liver was homogenized. To a series of 15 ml labeled tubes, 2 ml of each sample was added followed by addition of trichloroacetic acid for the precipitation of proteins. Following centrifugation for 10 mins, o-cresol, concentrated ammonium hydroxide and hydrogen peroxide were added subsequently. After 5 mins, all the tubes were inspected visually for the presence of purple color indica-

GT P-02

ting a positive result. For confirmatory analysis on GC-MS, all the samples containing PPD, and metabolites were alkalinized and extracted into a relatively non-polar solvent mixture. Extracts were derivatized with MTBSTFA and analyzed on an Agilent GC-MS SIM mode/ DB-5 MS capillary column. The analytes of interest were identified by their retention time and corresponding target ions (compared with retention time and target ions of the positive control).

Results & Discussion: PPD and/or its metabolites were detected in gastric contents of 66.6% of all PPD positive cases (120 total cases tested). Similarly, 23.3% cases were found positive both in blood and gastric contents for PPD and/or its metabolites. However, blood and liver counted 7.9% while liver and urine counted only for 2.2% for PPD and/or its metabolites detection of all poisoning (PPD) cases. Furthermore, detection of higher number of acetyl metabolites in gastric contents than in liver also indicates that PPD metabolism by acetyl transferases is greater in stomach than in liver.

Conclusion: The selection of most appropriate biological specimen in toxicological analysis is a critical step for the case under investigation. We are the first who reported that gastric contents are of utmost significance for PPD and its metabolites. Our findings suggest that gastric contents must be considered as sample of choice for PPD detection in addition to other samples in all antemortem (gastric lavage) and postmortem cases for routine PPD poisoning analysis. Moreover, this will not only fulfill the forensic toxicological criteria of minimum two specimens tested but will also be beneficial when other specimens are unavailable.

GT P-03

Evaluation of Kura FINDEN® novel β-glucuronidases using LC-MS/MS

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Background & Aims: The hydrolysis of glucuronides is an important step in the field of drug testing as this allows the conversion of conjugated drugs and metabolites back to their parent form, ensuring accurate drug detection and a streamlined workflow. Glucuronides are formed from the metabolism of toxic substances in the liver and are excreted in the urine. In order to split off the glucuronic acid and have the free substance available, the samples are hydrolyzed and detected through LC-MS/MS1 . Kura Finden® designed enzymes for a fast and simple cleavage of these metabolites. The recombinant β-glucuronidases B-One and BGTurbo as well as the recombinant β-glucuronidase/arylsulfatase mix BGS cleave the glucuronic acid from the substance within a few minutes at room temperature. The two previously used native β-glucuronidases in the routine are of native origin and are obtained from Helix pomatia. Both are used for 120 min at 55°C to split off the glucuronic acid.

1 Used equipment: ACQUITY TQD UPLC/MS system with ACQUITY UPLC HSS C18 columns

Methods: All five enzymes were compared to accurately determine which enzyme performed the best. The hydrolysis step varied in incubation time, incubation temperature and the amount of enzyme. The selected glucuronides were Oxazepam-glucuronide, THC-COOH-glucuronide and Buprenorphine-glucuronide. The chosen marker for a successful hydrolysis was a residual glucuronide content of 5%.

Results & Discussion: The results of the varied incubation times showed that all recombinant enzymes reached a residual glucuronide content of less than 5% in 10 minutes. The native β-glucuronidases needed 10 minutes to reach a residual glucuronide content of less than 5% for the Oxazepam- and THC-COOH-glucuronide. For the Buprenorphine-glucuronide they needed 30 minutes to fall below the limit of 5%.

Secondly, the incubation temperature was varied. All recombinant enzymes could perform the hydrolysis within 10 minutes at room temperature to achieve a residual glucuronide content of less than 5%. The native β-glucuronidases reached a residual glucuronide content of 5% within 30 minutes for the Oxazepam and THC-COOH-glucuronide at room temperature however, for the Buprenorphineglucuronide a temperature of 55°C was needed.

Lastly, the amount of enzyme was varied. All recombinant enzymes reached a residual glucuronide content of 5% within 10 minutes at room temperature using only half or even one quarter of the original enzyme volume. The glucuronides of Oxazepam and THC-COOH can also be hydrolyzed to a 5% residual content at 55°C in 30 minutes using half or one quarter of the original volume of native enzymes. A residual glucuronide content of 5% cannot be reached with the native enzymes for the Buprenorphine-glucuronide with half or a quarter of the enzyme volume.

Conclusion: Using Kura Finden® products, we were able to reach our goal of improving our current drugs of abuse screening method without investing in new equipment. All recombinant enzymes show similar efficiencies in the step of hydrolysis. The reason for incorporating B-One in the routine of MVZ Labor Dr. Limbach & Kollegen is that B-One is the most cost effective per batch and comes ready to use in its own buffer. By using the recombinant ß-glucuronidase B-One, we have been able to make our current screening method more sensitive. B-One has enabled us to improve our method while reducing costs in the process. Furthermore, the simple conditions for an efficient hydrolysis with B-One will simplify the step of automatization in the future.

Comparison of immunoassay based toxicology screening to liquid chromatography-time of flight/mass spectrometry (LC-ToF/MS) approaches.

GT P-04

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Background & Aims: Laboratories and death investigation agencies have limited resources in terms of drug screening approaches. The purpose of this study was to evaluate the effectiveness of a defined immunoassay screening panel versus screening by liquid chromatography/time of flight mass spectrometry (LCTOFMS), and to assess the significance of the positive findings from the non-targeted testing methodology which also allows for data mining, or secondary database interrogation. Both approaches however require confirmatory testing by an independent analytical method.

Professional Organizations such as the Organization of Scientific Area Committees (OSAC), American National Standards Institute's National Accreditation Board (ANAB), the National Safety Council (NSC), AAFS/SOFT, and others recommend or require confirmatory toxicological testing as a best practice in forensic toxicology, and it is required for accreditation purposes under standards endorsed by the National Association of Medical Examiners (NAME), the American Board of Forensic Toxicologists (ABFT) and others. Basic postmortem testing often utilizes presumptive immunoassay screening, which only covers categories of routinely misused drugs, and may lack sensitivity and specificity. The laboratory based screen and confirmation approach typically uses more sensitive and specific platforms, such as liquid chromatography/time of flight mass spectrometry (LC-TOF/MS), to identify a broader scope of compounds of toxicological interest than is feasible with immunoassay testing, and meet the requirements for forensic identification. We describe a comparison of immunoassay testing using ELISA technologies to an LC-TOF/MS screening approach coupled with Liquid Chromatography/tandem mass spectrometry (LC-MSMS) confirmatory testing.

Methods: We compared data from 618 death investigation cases in which an ELISA immunoassay testing protocol was compared to an LC-TOF/MS screening and confirmation. Basic immunoassay postmortem drug screening included twelve categories of routinely misused drugs; amphetamines, barbiturates, benzodiazepines, buprenorphine, cannabinoids, cocaine, fentanyl, methadone, methamphetamine/MDMA, opiates, oxycodone/oxymorphone, and phencyclidine. The comprehensive screen performed by LC-TOF/MS included more than 230 substances, including therapeutic and abused drugs, adulterants, antipsychotics, antidepressants, muscle relaxers, cardiovascular medications, pain relievers, hypnotic sedatives, and a selection of prominent novel psychoactive substances (NPS).

Results & Discussion: In 354 cases (57%) one or more additional substances of forensic significance not detected by immunoassay were detected in the LC-TOF.MS screen. An additional 1,965 compounds were confirmed in these cases by LC-TOF/MS screen for an average of 5.5 additional findings per case, not identified by immunoassay. This is indicative of the complexity of modern drug markets and the polydrug nature of today's opioid epidemic. This is made even more clear from seized drug testing which has confirmed that submitted exhibits usually contain more than one active substance, and in many cases more that 8, within individual exhibits, a characteristic often seen with illicit "dope" submissions and counterfeit pharmaceuticals. For example, para-fluorofentanyl, xylazine, and carfentanil are frequently present in some fentanyl positive cases that would not be discernable by immunoassay. In total, in this cohort, the additional findings identified through the LC-TOF/MS test comprised 114 drugs not identified in the basic immunoassay testing.

Another advantage of laboratory-based LC-TOF/MS is the ability to re-interrogate the LC-TOF/MS data for the presence of later emerging novel psychoactive drugs, especially novel synthetic opioids, designer benzodiazepines, novel synthetic stimulants, toxic adulterants, and new therapeutic drugs, broadening the insights into drug caused and related death. Nine different NPS were reported after LC-TOF/MS screening in this case series, with the designer benzodiazepine bromazolam accounting for 54% of the findings, and novel synthetic opioids, including metonitazene and protonitazene, accounting for another 18%.

Conclusion: Immunoassay screening in postmortem toxicology is an acceptable first approach to toxicology screening but comprehensive LC-TOF/MS provides a much broader scope and improves the quality of toxicology support for medicolegal death investigations and allows for additional data mining for new and emerging drugs which are not readily addressed in immunoassay screening approaches. When resources for postmortem toxicology are limited, it is advisable to consider the case history and case specific information that may favor one approach over the other.

Differential cannabinoid-like effects and pharmacokinetics of ADB-BICA, ADB-BINACA, ADB-4en-PINACA and MDMB-4en-PINACA in mice

GT P-05

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Background & Aims: Synthetic cannabinoids (SCs) have gained popularity in many countries over the past decade as an alternative to natural cannabis. As of 31 December 2021, the European Monitoring Center for Drugs and Drug Addiction (EMCDDA) has been tracking 224 SCs that have surfaced on the drug market since their initial identification in 2008. Importantly, the widespread misuse of SCs worldwide has precipitated a global public health crisis. Despite synthetic SCs prevalent use among humans, these substances often lack comprehensive pharmacological data, primarily due to their rapid emergence in the market. This study aimed to discern differences and causal factors among four SCs (ADB-BICA, ADB-BINACA, ADB-4en-PINACA and MDMB-4en-PINACA), with respect to locomotor activity, body temperature and nociception threshold.

Methods: Motor function changes were assessed through spontaneous locomotor activity and homecage behavior tests. A digital rectal thermometer with a lubricated flexible probe was used to measure body temperature. The thermal nociceptive threshold was assessed using the hot plate test. Molecular docking was employed to investigate the binding interactions of the four drugs with CB1R. Additionally, LC-MS/MS analysis was utilized to track changes in plasma concentrations of the four drugs at varying time points post-administration.

Results & Discussion: Adult male C57BL/6 mice received intraperitoneal injections of varying doses (0.5, 0.1 and 0.02 mg/kg) of these compounds. Three substances (including ADB-BINACA, ADB-4enPINACA and MDMB-4en-PINACA) demonstrated dose- and time-dependent hypolocomotive and hypothermic effects. Notably, 0.1 mg/kg MDMB-4en-PINACA exhibited analgesic properties. However, ADB-BICA did not cause any effects. MDMB-4enPINACA manifested the most potent and sustained effects, followed by ADB-4enPINACA, ADB-BINACA and ADB-BICA. Additionally, the cannabinoid receptor 1 (CB1R) antagonist AM251 suppressed the effects induced by acute administration of the substances. Analysis of molecular binding configurations revealed that the four SCs adopted a congruent C-shaped geometry, with shared linker binding pockets conducive to robust steric interaction with CB1R. Essential residues PHE268 , PHE200 and SER173 within CB1R were identified as pivotal contributors to enhancing receptor–ligand associations. During LC-MS/MS analysis, 0.5 mg/kg MDMB-4en-PINACA exhibited the highest plasma concentration and most prolonged detection window post-administration. The study of SCs' pharmacological and pharmacokinetic profiles is crucial for better understanding the main mechanisms of cannabinoid-like effects induced by SCs, interpreting clinical findings related to SC uses and enhancing SCs risk awareness.

Conclusion: In this study, we compared the differences in cannabinoid-like effects induced by four synthetic cannabinoids in mice. For four drugs, MDMB-4en-PINACA manifested the most potent and sustained effects, followed by ADB-4en-PINACA, ADB-BINACA and ADB-BICA. Although we explicated the causes for the differences from the perspectives of pharmacology, molecular interaction and pharmacokinetics, the mechanism for the differences have not been thoroughly explored. Therefore, a more comprehensive exploration including downstream molecular signal, in vivo distribution of drugs and key interacting residues is warranted in future research to elucidate the nuances in both pharmacology and pharmacokinetics.

GT P-06

Unveiling the toxicity of synthetic cannabinoids: Insights from behavioral and molecular studies in vivo and vitro

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Background & Aims: Synthetic cannabinoids (SCs) are the most widely used novel psychoactive substances (NPS) and pose significant medical and psychiatric risks worldwide. Due to the structural diversity and rapid prevalence of synthetic cannabinoids (SCs) in the market, the information linking the chemical structure of SCs to their toxicity remains scant. In the present study, we aimed to investigate the toxicity and underlying mechanisms of indole synthetic cannabinoids JWH-018 and JWH-019 in mice and modified human embryonic kidney (HEK) 293T cells, using behavioral, pharmacokinetic, pharmacological approaches, and molecular docking.

Methods: Adult male C57BL/6 mice (6-8 weeks old) were used as animal model. Cannabinoid-like effects including hypolocomotion, hypothermia, and analgesia were evaluated in mice following intraperitoneal administration of JWH-018 and JWH-019. The motor function changes were detected through two test methods, spontaneous locomotor activity and homecage behaviors. The core body temperature was measured using a digital rectal thermometer with a lubricated flexible probe.The hot plate test was performed to assess analgesia effect. To determine if these effects were mediated by cannabinoid receptor 1 (CB1R) or 2 (CB2R) activation, we administered CB1R and CB2R antagonists (AM251 and AM630) before drug administration. Additionally, LC-MS/MS analysis tracked the concentration changes of both drugs in mouse plasma at various time points after administration. We also evaluated the ERK1/2 pathway, a downstream signaling molecule of CB1R, in both live mice (in vivo) and cultured cells (in vitro). Finally, molecular docking was used to investigate how these drugs interact with CB1R.

Results & Discussion: Cannabinoid-like effects, including hypolocomotion, hypothermia, catalepsy, and analgesia, manifested in four phenotypes, represent a common preclinical model in rodents to evaluate whether a pharmacological compound acts as a CB1R agonist. In the present study, JWH-018 induced dose-and time-dependent hypolocomotion, hypothermia, and analgesia, while JWH-019 did not produce any of these effects at the doses tested. JWH-018 and JWH-019, based on the structural model proposed by EMCDDA, share both the naphthyl head group and the indole core group. The key structural difference between these two cannabinoids lies in the length of their tail alkyl chains. The underlying mechanisms and reasons for the observed differences in biological effects between these two drugs remain unreported.

Accumulating evidence suggests that activation of CB1R plays a central role in mediating the pharmacological and behavioral effects of most cannabinoids. In our study, pre-administration of the CB1R antagonist AM251 reversed the cannabinoid-like effects induced by JWH-018, while AM630, a CB2R antagonist, did not. These findings highlight the critical role of CB1 receptors, as opposed to CB2 receptors, in mediating the effects of JWH-018 in mice.

Upon CB1R stimulation, mitogen-activated protein kinases (MAPKs), including extracellular signal-regulated kinases (ERK1/2) can be activated, triggering additional cellular effects. To explore the mechanism underlying the differences in cannabinoid-like effects between the two drugs, the levels of total ERK1/2 and phosphorylated ERK1/2 (p-ERK1/2) were analyzed in vivo and vitro. In vivo, JWH-018 administration significantly increased the phosphorylation of ERK1/2 compared to the control group in the thalamus, hippocampus, cortex, and cerebellum 30 minutes after drug administration at a dose of 0.5 mg/kg. In contrast, JWH-019 did not induce ERK1/2 phosphorylation in any of these brain regions. Moreover, pre-treatment with AM251 significantly blocked the JWH-018-induced phosphorylation of ERK1/2 in the thalamus and hippocampus. In vitro, HEK293T cells with stable CB1R expression and control cells were exposed to 1 μM of each drug, and the ratio of phosphorylated ERK1/2 (p-ERK1/2) to total protein was analyzed after 6 minutes. In control cells, neither drug caused significant ERK1/2 phosphorylation. In contrast, JWH-018, but not JWH-019, induced ERK pathway activation in HEK293T cells stably expressing CB1R.

The blood concentrations of both drugs followed a similar pattern within the 3-hour timeframe at a dose of 0.5 mg/kg, with peak concentrations observed 30 minutes after administration (75.25 ng/mL for JWH-018 and 98.15 ng/mL for JWH-019). The concentrations then decreased rapidly, with very low levels detected at 3 hours.

A comprehensive molecular docking analysis was undertaken to elucidate the structural-activity mechanisms underlying the interactions between the four drugs and CB1R. Both JWH-018 and JWH-019 adopt a similar C-shaped binding pose within the binding pocket of the CB1R complex. This pocket is formed by transmembrane helices 2, 3, 5, and 7 and capped by the ECL2 loop. Docking simulations revealed the formation of π-π interactions between the naphthyl head group of both JWH-018 and JWH-019 and the TRP279 residue of CB1R. Additionally, π-π interactions were observed between the indole core of both cannabinoids and PHE268 and PHE170 of CB1R.

Conclusion: The difference of cannabinoid-like effects induced by JWH-018 and JWH-019 was mainly related to downstream molecular signal abnormalities mediated by CB1R. These findings shed light on the molecular pharmacology and activation mechanism of SCs for CB1R and should guide further research into the mechanisms underlying their deleterious effects in humans.

When solitary sexual activity turns into drama. How to detect "poppers" use in post-mortem case?

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GT P-07

Background & Aims: It is in a context of solitary sexual practice that a man is found dead: he is wearing sadomasochistic clothing, white tablets and bottles of poppers are found near him. A forensic doctor carried out a body examination, no autopsy was carried out. He concludes to a very marked agonal syndrome that is not specific to a cause of death and a reference toxicological assessment is carried out. The toxicological results were negative regarding the search for volatile substances. This case study discusses the difficulties of revealing, through toxicological analyses, the use of poppers.

Methods: Following the body examination, a toxicological expertise was carried out on peripheral blood and urine samples. Search and quantification of alcohols (ethanol, methanol and acetone) were carried out in blood and urine by gas chromatography with flame ionization detection after injection by the headspace system. Narcotics and medications were detected in the blood by enzyme immunoassay (ELISA®). A broad toxicological screening was carried out in the blood by 2 distinct chromatographic methods combining LC-MS/MS (Waters Xevo TQD®) and LC-DAD. New psychoactive substances (NPS) were searched for in blood and urine by liquid chromatography after liquid-liquid extraction in an alkaline medium and detection by tandem mass spectrometry on a system (Waters

Xevo TQD®). Finally, a specific search for volatile substances was carried out on urine sampling by gas chromatography with detection by mass spectrometry after injection by the headspace system (Thermofisher Focus GC + DSQII®).

Results & Discussion: The scene of the body's discovery is evocative of an increasingly widespread practice: solitary or autoerotic sexual activity. Various cases of death resulting from this practice have been described in the literature: autoerotic death refers to accidental death during solitary sexual activity, caused by malfunction of the device used (asphyxia) or any other unforeseen event. Alkyl nitrites in particular act on the anal sphincter and vagina, facilitating penetration and significantly increasing sexual arousal. They delay ejaculation and prolong orgasm by slowing down temporal perception. Even if such a practice is preferentially centered on the populations of men who have sex with men, there seem to be signs of diffusion into other sub-populations (heterosexuals). What's more, the health crisis linked to the COVID-19 epidemic and the ensuing confinements have reinforced the practice of solitary Chemsex, with drug use during video-masturbation sessions.
No autopsy was performed, so the expert only had blood and urine samples at his disposal. The literature describes several cases in which metabolites of various volatile substances were detected in urine. However, volatile toxins are often introduced into the body via the respiratory tract, so they can be detected much more easily in the lung than in blood or urine. Thus, the analysis of organs such as the lung parenchyma, but also the brain, can be highly relevant. Both these organs are rich in lipids, making them ideal binding sites for many volatiles. Generally speaking, for all volatile products, the lung is the organ of choice for toxicological analysis, provided that an autopsy is performed soon after inhalation, and that the sample is placed in a perfectly sealed container. What's more, volatile compounds that have disappeared from other organs during the putrefactive liquefaction process may be found in the cranium, which is relatively airtight. The loss of volatile substances through evaporation at room temperature is one of the main causes of difficulties in detecting these substances. For this reason, it is essential that samples of these compounds, are taken under rigorous conditions to avoid partial or total loss during sampling. Only sampling by sealed glass or plastic syringe is suitable. It must be stocked in low-volume, gas-tight, well-sealed containers with a minimum of headspace: "headspace" vials for headspace analysis by gas chromatography, enabling identification but also the quantification necessary for interpretation. If body removal or autopsy is carried out quickly, samples are stored at samples are stored at +4 °C. In the event of delayed examination, freezing at -20 °C is essential.

Conclusion: The negative result of volatiles subtances researchs can be discussed on several points. On the one hand, the absence of an autopsy did not allow the expert to analyze the lungs, the organ of choice for the analysis of poppers. On the other hand, alkyl nitrites being very volatile at room temperature, the blood and urine samples could have been taken with waterproof syringes, in airtight packaging suitable for headspace analysis and kept frozen in order to to avoid any loss of molecules. And finally, the instability of alkyl nitrites and their metabolites in biological matrices causes their rapid disappearance, thus making them difficult to detect. It is therefore essential to work in conjunction with the forensic pathologist and/or investigators for good direction of the research and in order to have the appropriate samples, taken in the best possible conditions.

GT P-08

A rapid and comprehensive UPLC™ System-MS/MS method for the analysis of benzodiazepines in urine

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Background & Aims: Benzodiazepines are important forensic toxicology drugs which are widely prescribed for neurological and psychiatric disorders and are also highly abused. They can cause side effects such as drowsiness, fatigue, amnesia and have the potential of addiction. Benzodiazepines are abused to reinforce opioid euphoric effects, counteract the effects of stimulant abuse or to perpetrate drug-facilitated sexual assault. Since the mid-2000s, many new designer benzodiazepines have been reported and continue to emerge, many of which were initially not controlled internationally. The potency and risks associated with designer benzodiazepines are largely unknown, causing public health and safety concerns. A particular concern is the growing use of designer benzodiazepines to make counterfeit tablets of commonly prescribed benzodiazepine medication, which can be unknowingly taken.

The aim of this study is to develop a comprehensive method for confirmatory analysis of traditional and designer benzodiazepines with UPLC System-MS/MS which includes a rapid and simplified mixed-mode sample preparation method.

Methods: Drug-free urine samples were spiked with traditional, e.g. diazepam, alprazolam and clonazepam, and designer benzodiazepines, e.g. etizolam, triazolam and midazolam. A seven-point calibration curve and quality control samples (QCs) at low, medium and high concentrations were prepared. One hundred microlitres of urine samples, calibrators and QCs were added to the wells of a Waters™ Oasis™ MCX µElution™ plate (Waters™) follo-

wed by 100 µL of a solution containing hydrolysis buffer and internal standards, and mixed by aspiration. The sample was loaded onto the sorbent bed by vacuum and subsequently washed with 200 µL of 80:20 water:methanol. After drying under high vacuum for one minute, the samples were eluted using 2 x 25 µL of 50:50 acetonitrile:methanol containing 5% strong ammonia solution. All samples were diluted with 150 µL of 2% acetonitrile: 1% formic acid prior to analysis.

A UPLC System-MS/MS method was applied using an ACQUITY™ UPLC™ I-Class PLUS System in conjunction with a Xevo™ TQ-S micro Mass Spectrometer operated in electrospray positive ion mode. Using an ACQUITY UPLC BEH™ C18 Column, all compounds were separated using a gradient elution profile from 2% mobile phase B (0.1% formic acid in acetonitrile) to 90% over 3.5 minutes before re-equilibration. Two MRM transitions were monitored for each analyte and a single transition monitored for the ISTDs (not all compounds analysed had a corresponding stable isotope labeled internal standard). Results of the developed method were assessed for recovery, matrix effects, accuracy, precision and linearity.

Results & Discussion: Care was taken during the development of the chromatographic conditions to ensure that compounds and internal standards did not interfere with each other, therefore, clonazepam-d4 was not used as an internal standard as it interfered with the quantitation of lorazepam. The panel of compounds chromatographed over 3.5 minute gradient elution, with retention times ranging from 1.55 to 3.26 minutes, with excellent separation of each compound and baseline resolution was achieved for the majority of compounds.

The mean extraction recovery average was 90.2% (range 68.3% to 110.8%), with the majority within the acceptable recovery range ± 20% (80-120%) for the entire panel of compounds in 6 different lots of urine, except for 8-aminoclonazepam (68%). Matrix effects were also evaluated using multiple lots of urine. As with recovery, matrix effects are essential for accurate quantitation. Ion suppression was observed for most analytes, the absolute matrix effects ranged from 13.7% to -32.9%, with the majority minimized to within ± 20% when corrected with an internal standard.

Calibration curves were prepared and analysed over the range of 5ng/mL to 1000ng/mL with coefficient of determination (r2) greater than 0.99 over 5 days of testing. Coefficients of variation (%CV) were less than 15% for all quantitative compounds tested at 4 concentrations over 5 days. Results for proficiency samples showed excellent agreement with the existing procedures.

Following the success of the developed method for the analysis of benzodiazepines in urine samples, further work is planned to assess whether the method is also suitable for other toxicologically relevant matrices, including whole blood.

Conclusion: The sample preparation method described was found to efficiently extract benzodiazepines and designer benzodiazepines from urine samples. The use of the ACQUITY UPLC BEH C18 C Column results in rapid analysis of a large panel of benzodiazepines while maintaining required baseline separations for accurate quantitation, whilst the Xevo TQ-S micro Mass Spectrometer ensures rapid and accurate quantitation of all compounds analysed over wide dynamic ranges. The combination of sample preparation, UPLC System separation and MS/MS detection results in a rapid, accurate and precise method for the comprehensive analysis of benzodiazepines in urine.

Was it really a crime? Challenges in toxicological analysis in Drug-Facilitated Sexual Assault Crimes

GT P-09

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Background & Aims: The laboratory of Forensic Chemistry and Toxicology of the Cyprus State General Laboratory, is the official government lab accredited to perform analysis of control drugs in seized materials, fire arms, explosives, GSR, clinical samples for emergency cases and toxicological analysis for medico-legal purposes.Drug-facilitated sexual assault (DFSA), is defined as a crime where the victim is subjected to sexual act while incapacitated due to alcohol or drugs impairment. As a result, the person is unable to react or consent to such acts(s). International listings of DFSA drugs are published by institutions such as the Society of Forensic Toxicologists (SOFT) or the UNODC. Our aim is to evaluate the results of the toxicological analysis from 34 samples submitted to our lab the period 2018-2023, in able to support the police and medical examiners to improve future investigation of DFSA crimes. Factors which complicate investigations include: " Lack of experience among investigators, medical personnel, laboratories and prosecutors in handling in DFSA cases'', ‘' lack of recognition of the crime by law enforcement agencies''. One of the major problems that toxicology labs face is the interpretation of the results related to the delays in reporting the incident. Proper sampling is also a major issue.

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Methods: After 2018, the lab has been equipped with two SCIEX 6500+ LC/MS/MS instruments, and highly-sensitive analytical methods were developed and accredited. Targeted Toxicological Analysis was performed on blood and urine for the determination of 120 substances.The range of the analyzing control drugs and prescription medicines has been expanded to include the majority of the listed substances.Liquid-Liquid extraction were applied and separation of the compounds was obtain on an Zorbax Eclipse Plus C18 column (2.1x50mm 1.8µm) with pre-column and gradient elution with A:5mmol/L ammonium formate pH 3,5 containing formiC acid and B :acetonitrile containing 0,1% formic acid. The lack of reference standards is a limitation for identification and confirmation for many of the NPS's and their metabolites. Alcohol was tested by Shimadzu QP 2010 HS-GC-FID.

Results & Discussion: In the reviewed cases, the time of the incident until reporting and sampling, ranged from 2-72 hrs. This leads to challenges for the detection of the drug and interpretation of the results in order to help legal authorities whether a crime has occurred. The victim's age range was 14- 81. All of the victims were female except one. 83% of the victims were tourists while being on holiday. Even though alcohol can be a major contributing factor and the most frequently detected substance when examine DFSA crimes, 26% of the examined samples (blood and urine) were positive for alcohol. Blood alcohol concentrations varied between 28mg/dl -81mg/dl at the time of the sample collection. Other substances detected, were diazepam, alprazolam, cocaine and metabolites, cannabis, methamphetamine, MDMA, MDA and antidepressants. 57% of the cases were positive for psychotropic substances and 30% were positive for medicines. All concentrations detected were relatively low. GHB (gamma-hydroxybutyric acid) screening in urine was negative.

Conclusion: DFSA crimes are characterized by delayed reporting of the incident by the victim, either due to the suppressive effects of the drugs or because of psychological stress. A standard protocol, for the medical examiners and police investigators will be applied to ensure that both blood and urine samples are properly collected as early as possible after the alleged DFSA been declared by the victim. Additional, is essential to collect as much contextual information as possible. Exhibits from the scene that could be analyzed, if available, can provide strong corroborative evidence. When urine is the only sample available, proper collection is of most importance for an accurate forensic interpretation of the analytical results (UKIAFT Guidelines for Alcohol Calculations). The complexity of DFSA cases and the analytical challenges necessitates well-trained scientists, sufficiently equipped laboratories, collaboration with medical examiners and police officers to provide the victims with the best justice.

A systematic toxicological analysis strategy for investigation of drug-facilitated sexual assault cases

GT P-10

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Background & Aims: In drug-facilitated sexual assault (DFSA), the victim is incapacitated due to either willing or covert consumption of intoxicating substances. The detection of a single drug exposure in investigation of DFSA requires rapid sample collection and a thorough systematic toxicological analysis strategy to find evidence of victim incapacitation. The aim of the project was to optimize the systematic toxicological analysis strategy for urine samples from DFSA cases by incorporating an enzymatic hydrolysis step into a fully automated sample preparation. Furthermore, the aim was to apply the method to urine samples from suspected DFSA cases and compare results with toxicological findings reported in DFSA cases worldwide.

Methods: A systematic toxicological analysis consisting of a fully robotic sample preparation with an automated enzymatic hydrolysis step and an UHPLC–MS/MS screening method was developed. On a Hamilton Microlab Vantage 2.0 liquid handling system, aliquots of 50 µl urine sample was enzymatically hydrolyzed with 50 µl recombinant enzyme for 5 min at room temperature followed by protein precipitation with acetonitrile. After evaporation and reconstitution, 2 µl was injected into the UHPLC-MS/MS system. An analyte panel of 144 drugs and metabolites was chosen based on recommendations by the Society of Forensic Toxicologist (SOFT) and additional drugs commonly found in Denmark. The UHPLC–MS/MS screening method was validated according to international guidelines for validation of qualitative methods including matrix effect, selectivity, carry-over, extraction recovery, and process efficiency. The screening method was applied to authentic urine samples from suspected DFSA cases (n=38), and results were compared to findings reported in scientific literature across the world.

Results & Discussion: An enzymatic hydrolysis step was implemented into a robotic automated sample preparation of urine samples. The automation enabled a simple and efficient workflow in which both urine and blood samples could be prepared and analyzed simultaneously. Successful enzymatic hydrolysis was achieved with an average parent drug recovery of 97%. The developed and validated UHPLC–MS/MS screening method covered at total of 144 drugs and metabolites relevant for DFSA with LODs ranging from 0.0001 mg/L to 2 mg/L and meeting the SOFT recommended minimum performance limits for most analytes. The method was applied to urine samples

from suspected DFSA cases and showed drugs of abuse (50%), benzodiazepines (29%), and antidepressants (24%) as prevalent substances. Toxicological reports from other countries showed similar findings with drugs of abuse, benzodiazepines, antidepressants, analgesics as substances commonly detected in suspected DFSA cases. Additionally, ethanol was one of the major reported substances in publications from other countries indicating alcohol consumption as a notable risk factor for DFSA.

Conclusion: The systematic toxicological analysis of urine samples from DFSA cases was improved with the incorporation of an enzymatic sample pretreatment into a robotic automated sample preparation and development of an UHPLC–MS/MS screening method. Application of the method showed a high level of drugs of abuse, benzodiazepines, and antidepressants in urine samples from suspected DFSA cases. Comparison to toxicological findings from DFSA cases reported worldwide showed that ethanol, drugs of abuse, benzodiazepines, antidepressants, and analgesics are often found in these cases.

GT P–11

Assessment of salivary thiocyanate level among active and passive smokers

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Background & Aims: Smoking is the most significant preventable epidemic faced by healthcare professionals and the global population. According to the latest estimates from the World Health Organization (WHO), there are over one billion smokers worldwide, accounting for 22.3% of the global population, with over 80% of them living in low- or middle-income countries, including Algeria. To study the issue of tobacco intoxication in smokers and help them quit smoking, it is necessary to measure smoking biomarkers in biological samples. There are two types of specific biological markers, such as nicotine, and non-specific markers, such as thiocyanate, which is produced from hydrogen cyanide and nitriles formed during tobacco combustion. The objective of our study is to evaluate the levels of salivary thiocyanates in active and passive smokers.

Methods: To achieve our objective, we carried out a dosage of saliva sample of 113 volunteers from 18 years old (adult subjects) of both sexes in the study, including 57 active smokers and 56 passive smokers. We included in the study:
Group 1: Active smokers: any subject who has consumed tobacco at least once a day, regardless of whatever the mode.
Group 2: Non-Smokers (passive smokers): People in contact with tobacco but not consumer

withdrawn

Samples of salivaby were analysed by spectrophotometric method using Spectrophotometer UV-VIS .
The reading of samples is taken at a wavelength of 400 nm.

Results & Discussion: The average level of salivary thiocyanates ions which is non-binding biomarker specific smoking habits in active smokers in our study is 0.20 ± 0.12 g/L. The rate of thiocyanate is independent of the age of active smokers. However, there is therefore a significant correlation between the rate salivary thiocyanate and duration of tobacco consumption($p = 0.000$, $p < 0.005$) and the number of cigarettes smoked per day ($p = 0.011$, $p < 0.05$). For a duration of tobacco consumption of more than 25 years, the average rate of The highest thiocyanate is 0.34 ± 0.15 g/L. In the case of consumption of 21 to 30 cigarettes per day, salivary thiocyanate levels are 0.24 ± 0.13 g/L .The average salivary thiocyanate level among passive smokers in our population is 0.09. ± 0.08 g/L, with a minimum concentration of 0 g/L and a maximum concentration of 0.47g/L. There is no significant correlation between the rate of salivary thiocyanates and duration of exposure either the intensity of exposure .

Conclusion: The concentrations of salivary thiocyanate ions, which is non-specific biomarker of smoking, can serve as a means to measure the smoker's response to anti-smoking therapy.

GT P–12

Exploring drink spiking and needling in the United Kingdom

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Background & Aims: Drink spiking, the act of introducing drugs or substances (including alcohol) into beverages or food to incapacitate individuals for various malicious purposes including sexual assaults, theft, pranks, or abuse, has become a concerning societal issue. In the UK, since October 2021, a new mode of drugging has emerged: needling, where the drug is introduced by needle injection. For drink spiking, testing kits have emerged as a potential solution, as they give the opportunity to test a drink which is suspected to be spiked. However, their reliability has been questioned.

The aim of our presentation is to investigate the prevalence, perception and experience of drink spiking and needling among the general public in the UK, as well as explore anti-spiking initiatives in the UK. We also aim at discussing the applicability and effectiveness of testing kits available in the UK (at the time of the study).

Methods: Two Drinkaware Monitor surveys conducted by YouGov in 2022 and 2023 gathered data from 10,473 and 6,318 respondents respectively, focusing on drink spiking/needling incidents. These surveys relied on self-reported experiences, perceptions, and the subsequent impact on individuals. In 2022, experience questions were not time-bound, whereas in 2023, questions asked about experiences in the last year.

Following the survey analysis, the study delved into preventive initiatives undertaken by establishments and venues in Cambridgeshire to address drink spiking/needling. Additionally, the efficacy of commercially available drink testing kits (All Test, Drink Safe, CYD, One Step, and Xantus; available in the UK at the time of study) was evaluated using standard drug solutions and mock spiked drinks. These findings were then substantiated by collecting drinks from clubs and bars and analysing them using both the drink testing kits and Gas Chromatography – Mass Spectrometry (GC-MS).

Two Drinkaware Monitor surveys conducted by YouGov in 2022 and 2023 gathered data from 10,473 and 6,318 respondents respectively, focusing on drink spiking/needling incidents. These surveys relied on self-reported experiences, perceptions, and the subsequent impact on individuals. In 2022, experience questions were not time-bound, whereas in 2023, questions asked about experiences in the last year.

Following the survey analysis, the study delved into preventive initiatives undertaken by establishments and venues in Cambridgeshire to address drink spiking/needling. Additionally, the efficacy of commercially available drink testing kits (All Test, Drink Safe, CYD, One Step, and Xantus; available in the UK at the time of study) was evaluated using standard drug solutions and mock spiked drinks. These findings were then substantiated by collecting drinks from clubs and bars and analysing them using both the drink testing kits and Gas Chromatography – Mass Spectrometry (GC-MS).

Results & Discussion: Our research found significant differences in reported drink spiking and needling incidents across two surveys (n=711 and n=89 in 2022, and n=231 and n=71 in 2023, respectively). More females reported being spiked than males, but the difference wasn't significant (three women for every two men in 2022; 2.3% women and 2.1% men in 2023). Bars (41%) and clubs (28%) remained the most common locations for drink spiking, while needling incidents slightly increased in clubs (24%) compared to bars (21%).

Cambridgeshire venues primarily relied on CCTV for prevention, followed by anti-spiking protections and entry searches. Implementing anti-spiking measures correlated with reported drink spiking instances. Immunoassay tests offered better reliability than color-based kits but had limited drug coverage, mainly GHB, ketamine, and benzodiazepines. However, both tests often failed to detect spiked levels of drugs. Instrumental analysis of drink samples from clubs and bars that tested positive showed no presence of the drugs studied (44 different drugs and pharmaceutical compounds).

Conclusion: Our research shows a significant prevalence of drink spiking compared to needling, with instances of drink spiking being approximately eight times higher overall. Over the past year, this gap has widened further, with drink spiking occurring three times more frequently than needling. Both drugging methods are followed by short- and long-term health complications, however, needling encompasses additional injection-specific health risks. While historically there has been a gender gap in drink spiking incidents, with three women spiked for every two men, the 2023 survey data suggests a more equal distribution between genders. These findings underscore the need for continued vigilance and targeted preventive measures, particularly in nightlife environments, to address and mitigate the risks associated with drink spiking and needling. In terms of preventative measures, venues' responses varied, with some inconsistency in reporting and prevention measures like CCTV and entry searches. Commercial testing kits showed limited reliability, and conventional testing methods failed to provide conclusive evidence of drug presence in drink samples. Addressing this issue will require multifaceted approaches involving public awareness, collaboration with establishments, and advancements in detection methods.

Genotoxicity of small-leaved linden infusion: A study on murine model using micronucleus test

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GT P-13

Background & Aims: Lime flower" is the English common name for " "Tiliae flos,," the flowering tops of the small-leaved lime tree,a plant widely used in traditional medicine for "Cough and cold, mental stress and mood disorders in the form of an infusion. This plant has been the subject of a monograph by the European Medicines Agency, which revealed many gaps in its safety profile, particularly its genotoxic (mutagenic) risk.

This study investigated the genotoxic effect of small-leaved linden infusion on a murine model using the micronucleus test, which can detect chromosomal lesions or mitotic apparatus of erythroblasts following the introduction of a test substance, resulting in the formation of micronuclei (circular fragments of chromosomes or whole chromosomes) in both immature polychromatic erythrocytes (PCE) and mature normochromatic erythrocytes (NCE).

Methods: Female albino mice were orally given the preparations to be tested, and divided into three groups (5 mice per group): negative control group (placebo), positive control group exposed to cyclophosphamide 16 mg/kg/day, and test group exposed to small-leaved linden infusion. After a 28-day exposure, samples were taken from all mice in the study, and peripheral blood smears were stained with an optimized MGG stain and analyzed for the detection of micronuclei in PCE and NCE erythrocytes. The enumeration of micronucleated cells was done on a population of 2000 cells of each erythrocyte type (PCE and NCE), and a ratio of PCE to NCE was established for each sample

Results & Discussion: The results showed that the group treated with cyclophosphamide had a significant and statistically significant increase in the mean number of micronucleated cells in the populations of NCE and PCE (15.2 and 40.6/2,000, respectively) compared to the placebo group (3.2 and 4.2/2,000, respectively). Small-leaved linden infusion, with a rate of micronucleated cells of 3.2/2,000 for NCE and 4/2,000 for PCE, did not show a significant difference compared to the placebo results. The calculated mean PCE/NCE percentages were: 1. negative control group (placebo): 1.9%, 2. positive control group (cyclophosphamide): 2.4%, and 3. test group (small-leaved linden): 0.92%.

While the results show no genotoxic effect of small-leaved linden, it was surprising to note a decrease of more than 50% in PCE (immature erythrocytes) compared to the populations of NCE (mature) in mice treated with the infusion compared to those who received the placebo. This result may constitute an indication of a potential myelosuppressive effect affecting the erythrocyte lineage.

Conclusion: In conclusion, a thorough study of bone marrow samples is necessary to confirm the ability of small-leaved linden to interfere with the hematopoietic process

GT P–14

Determination of lambda cyhalothrin poisoning in aerobatic pigeons using gas schromatography-mass spectrometer

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Background & Aims: Lambda cyhalothrin is a synthetic pyrethroid insecticide used worldwide in agriculture, protection of disease vector control and home pest control. Its molecular weight is 449.850 amu with molecular formula C23H19ClF3NO3. Its mode of action reveals that it affects nervous system by disrupting the gating mechanism of sodium channels involved in nerve impulses. Its toxicological effect causes paralysis or death in victim organisms. In Pakistan, its use in many homicidal and suicidal cases has also been reported. In current case study, an incident of death of one hundred aerobatic pigeons owned by a local bettor of Dera Ghazi Khan, a district of South Punjab Province, Pakistan, has been discussed. The bettor used these pigeons in flying competitions of birds, which is a famous birds' sport in Pakistan. Such aerobatic pigeons are considered to be very expensive and are kept in cages under special care. In a morning, the bettor found all of one hundred pigeons dead in their cages. He had suspicion that a paid worker, whose duty was to take care of pigeons' feed and health, had given some poison to pigeons mixed in feed. This forensic toxicology case was submitted in clinical and forensic toxicology lab in 2023 to determine cause of death. Gizzards of all one hundred pigeons, feed and water samples were collected and sent for toxicological analysis to determine the cause of pigeons' sudden collective death.

Methods: Dissection of one hundred dead pigeons was performed in a local veterinary hospital by authorized doctor. Gizzard samples of all one hundred pigeons were collected (preserved in normal saline) and sent to toxicology lab. Ten gram homogenized feed sample was collected from each feed container (total ten containers were placed in cages). One hundred ml water sample was collected from a single water reservoir designed for water supply to all cages. In toxicology lab, gizzard samples were grinded and homogenized in deionized water (1:1). Pigeons' feed was apparently comprised of pearl millet grains and it was just grinded in dry condition. Drinking water of pigeons was processed as it is. Samples' extraction was performed with modified dispersive solid-phase extraction method using acetonitrile solvent and anhydrous MgSO4 at 9 pH. Analysis of samples was performed on GC-MS equipped with electron impact ionization source using a pre-set validated method for simultaneous quantification of nine multi-

class pesticides including lambda-cyhalothrin. Analytical method on GC-MS was validated for the quantification of permethrin, lambda cyhalothrin, pyriproxyfen, triazophos, profenophos, chlorpyriphos, carbofuran, phorate and sulfotep. A GC system (7890B), auto-injector, autosampler coupled with Inert MS system (5977B) of Agilent Technologies were used as an analytical tool. Enhanced Mass Hunter Software was used to operate GC-MS. Injection volume was 2 µL set in the method using 5 µL micro-syringe. The split-less mode of the inlet was operated at 250 °C. Wall coated open tubular DB-35 ms capillary column (film thickness 0.25 µm × internal diameter 0.25 mm × column length 15 m) of Agilent Technologies was used in the GC system. Helium was used as carrier gas at constant pressure mode. Temperature programming of GC system includes 100 °C initially with 0.5 min hold-time. Then the temperature was raised to 300 °C at the rate of 20 °C/min, hold time 3.5 min, the total run time of 14 min. The temperature of the MS transfer line was 280 °C. EI at the voltage of 70 eV was applied for ionization purposes. The temperatures of the ionization chamber and mass analyzer were set to 300 °C and 150 °C respectively. For quantification of analytes, the SIM model was applied. Three ions selected for lambda cyhalothrin were m/z 181.1, 141.1 and 180.3.

Results & Discussion: All gizzard samples of one hundred pigeons were positive for lambda-cyhalothrin and quantity range was 0.05-0.08 ng/g. All ten feed samples were positive for lambda-cyhalothrin and quantity range was 0.27-0.43 ng/g. While the water sample was also positive for lambda-cyhalothrin and the quantity detected was 0.25 ng/mL.

Conclusion: After obtaining toxicological examination results, it was interpreted that all victim pigeons consumed feed and water that were contaminated with lambda-cyhalothrin by some culprit. The mystery was solved when toxicology report was shared with the owner of dead pigeons, he captured the suspect worker who confessed later on that he had given poison to the pigeons in jealousy. Selected ion monitoring mode confirmed the presence of mentioned pesticide with true quantification using
GC-MS. So, it was concluded that lambda-cyhalothrin was cause of death for one hundred pigeons as its presence in gizzards, feed and water supported the toxicological interpretation.

Scopolamine detection in subjects treated with N-butylscopolamine: Toxicologic considerations in DFC and DFSA cases

GT P-15

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Background & Aims: Scopolamine is a tropane alkaloid present in many plants of the Solanaceae family. It possesses antimuscarinic activity at the central nervous system level as well as throughout the body. In the forensic lab context scopolamine is often investigated in cases where a drug facilitated crime (DFC) scenario is suspected, including drug facilitated sex assault (DFSA), as it may cause confusion, agitation, delirium, anterograde amnesia and submissive behavior. However, based on our experience and on the consulted literature, the actual detection of scopolamine related to these cases is anecdotal. On the other side, N-butylscopolamine is a frequently used antispasmodic drug generally used for the symptomatic treatment of abdominal cramping and pain.

The finding of scopolamine in a DFSA oriented case in our lab and the subsequent detection of N-butylscopolamine raised a red flag about the correct interpretation of the results in these cases. The aim of this work was to determine the most adequate lab protocol specifically for DFC/DFSA oriented cases where scopolamine is found.

Methods: Blood and urine samples of a 33-year-old female with a medical history of breast cancer and possible victim of a DFSA in a restaurant were received in our lab. The time interval between the alleged events and the sample collection was approximately 18 hours.

Samples were initially processed following our standard lab procedure for general toxicological screening, including enzyme immunoassay (CEDIA), ethanol determination and a solid phase extraction (SPE) before analysis with a UHPLC-HRMS-Q-Orbitrap system as well as HPLC-DAD and GC-MS.

After the initial findings, the remaining urine sample was diluted and directly analysed with a LC/MS QTOF system. A blood sample submitted to ultrafiltration was also additionally investigated by UHPLC-HRMS-Q-Orbitrap.

Results & Discussion: The immunoassay screening was negative. No ethanol was detected in blood and 0.14 g/L ethanol was detected in urine. Traces of scopolamine as well as metoclopramide and its metabolites were detected in the SPE extracted urine sample. When the remaining urine sample was pretreated using a dilution protocol, N-butylscopolamine was additionally detected. The proportion of scopolamine to N-butylscopolamine based on the areas was approximately 1:400.

Traces of metoclopramide were detected in the SPE extracted blood sample, but neither scopolamine nor N-butylscopolamine were found. However, after ultrafiltration of the blood sample, traces of N-butylscopolamine were detected.

Conclusion: In cases where scopolamine is detected, especially if DFC is suspected, the presence of N-Butylscopolamine should be specifically investigated as the finding of the former might be associated with the presence of the latter. However, due to its chemical nature N-Butylscopolamine might escape protocols were only SPE is routinely performed. Thus, a protocol including dilution or ultrafiltration pretreatments combined with SPE may be of choice in these cases to avoid possible misinterpretation of the results. Our lab is currently investigating the origin of scopolamine traces associated with the presence of N-butylscopolamine.

GT P–16

Rapid seized drug analysis by RADIAN ASAP MS and confirmation by high resolution mass spectrometry

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Background & Aims: Illicit drug use and trafficking causes harm, instability, and violence; the analysis of seized drugs plays a vital role in the effectiveness of national and international programs which aim to control the use, trafficking and distribution of illegal drug substances. The increased number of samples seized and submitted for analysis along with diversity and potential toxicity, places a huge burden on drug control and forensic laboratories to produce reliable results quickly. Consequently, analytical methods that provide fast, reliable results are of interest.

The aim of this study was to assess the potential of a compact device based on Atmospheric Solids Analysis Probe-Mass Spectrometry (RADIAN™ ASAP MS), as a simple, rapid screening tool for the detection of drug substances in seized materials and subsequent confirmatory analysis using an established UHPLC-TOF-MS method.

Methods: A number of suspect materials (n=229) confiscated at music events/night-time venues were supplied by the UK police. The seized samples (pills, capsules and powders) were split into 23 groups based on seizure and sample appearance.

The seized materials were fully dissolved into 5 mL of methanol (stock), diluted 1:20 with methanol and sampled by dipping a glass capillary into each dilution prior to analysis by RADIAN ASAP MS. Mass detection was performed using full scan MS (m/z 50-650), at 600°C with an analysis time of approximately 30 seconds. Data were acquired simultaneously at four cone voltages (15, 25, 35, 50 V), which generated characteristic precursor and fragment ions. Data were processed using LiveID™ Software and compared with the seized drug spectral library (Waters) for 79 analytes. A minimum average match score of 850 (from a maximum 1000) was used as the reporting criteria for a positive detection.

Confirmatory analysis was performed using the Waters ACQUITY™ UHPLC™ I-Class System combined with a Xevo™ G3 QTof Mass Spectrometry (UHPLC-TOF-MS) to assess the performance of the RADIAN ASAP MS. The seized sample stock solutions were diluted, 1:2,000 with 5mM ammonium formate pH 3.0, prior to screening with the UHPLC-TOF-MS. Chromatographic separation was achieved in 15 min and data were acquired using MSE acquisition mode. Identification was based on retention time (±0.35 min of reference retention time), detection of a precursor mass, and the presence of at least one diagnostic fragment ion. Data were compared with an established toxicology library (Waters) based on retention time and accurate mass fragment data for >2000 analytes.

In addition, semi-quantitative analysis using RADIAN ASAP MS was evaluated on a sub-set of the seized material identified as containing cocaine and MDMA. A solvent calibration series (5 µg/mL – 1000 µg/mL) was prepared for cocaine and MDMA, using certified reference materials in methanol containing an internal standard (ISTD molsidomine at 25 µg/mL). The seized sample stock solutions were diluted with methanol containing the ISTD (molsidomine at 25 µg/mL). The prepared standards and samples were subsequently analysed following the acquisition procedure previously described.

Results & Discussion: RADIAN ASAP MS analysis of the 229 seized samples led to the positive detections of one or more compounds, when matched to the RADIAN ASAP MS seized drug spectral library. A total of 252 positive identifications were made: MDMA (74%), flualprazolam (9.5%), cocaine (7%), caffeine (4%), paracetamol (4%), etizolam (2%) and amphetamine (0.5%) with average match scores ranging from 898 to 993. Overall the RADIAN ASAP MS demonstrated excellent qualitative agreement with the UHPLC-TOF-MS analysis, confirming the 252 positive identifications. The UHPLC-TOF-MS confirmatory analysis also detected additional compounds to the RADIAN ASAP MS screen, which is likely due to the expanded UHPLC-TOF-MS library content and increased analytical sensitivity.

Semi-quantitative evaluation using RADIAN ASAP MS on a sub-set of seized samples for cocaine and MDMA, detected analyte concentrations ranging between 78mg to 170mg for cocaine, and 18mg to 50mg for MDMA.

Conclusion: RADIAN ASAP MS is compact device which provides a rapid and easy-to-use screen that can identify single or multiple components in seized materials. The simple workflow has demonstrated consistency and confidence in identification of compounds across pills, powders and capsular seized samples.

Factors favoring the migration of antimony into bottled water

GT P–17

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Background & Aims: The consumption of bottled water has increased significantly in recent times. Polyethylene terephthalate (PET) is the material used in the packaging of these waters. However, many studies have linked PET to antimony. The objective of our study was to evaluate the influence of time storage and temperature on the migration of antimony from PET to water

Methods: This is a cross-sectional study for analytical purposes conducted on six brands of bottled water sold on the Algerian market. After immediate analysis, the samples were stored under different conditions of time (10 and 30 days) and temperatures (2°C, 25°C.40°C). The analysis was carried out by polarography

Results & Discussion: The results revealed that the average level of antimony increased from 0.5µg/L before storage to 1.49 µg/L after storage at 40°C for one month for the 1.5L bottles and from 0.7µg/L to 2.34µg/L for 0.5L bottles. Sb levels were higher in the 0.5L bottles than in the 1.5L bottles without exceeding the recommended limits. A significant positive correlation between Sb level and time and temperature was noted.

Conclusion: The values of the estimated daily intake (EDI) were well within the norms, which means that its consumption does not present any risk to human health

Impact of smoking on serum vitamin C levels: A comparative study

GT P–18

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Background & Aims: Cigarette smoke is a significant source of oxidative stress, and many studies have demonstrated that heavy smoking in males is associated with a decrease in serum ascorbic acid (AA) levels. The objective of this study is the determination of AA levels in a group of smokers in a comparison with a control of non-smokers

Methods: Our study was carried out on a sample of 62 subjects (30 non smokers's and 32 smoker's person) with an average age of 33.62 years old and an average BMI of 23.62 kg/m2

41% of smoker's group consume less than 16 cigarette a day, only 21% take more than 25 cigarette

Serum vitamin C levels of 62 people were analyzed via a spectrophotometric method. The maximum absorption of the color developed by the interaction of AA with Folin reagent is 769 nm. 4 mL of venous blood was collected from each individual into citrate tubes; serum samples were separated to be conserved at 4°C for vitamin C determination.

Results & Discussion: The average of vitamin C for smokers and non-smokers were respectively 8.62 mg/L and 9.62 mg/L. This difference was statistically significant ($p < 0.000$). Also, we noted a significant negative correlation between both serum level of vitamin C in the smokers' study group and the number of cigarettes consumed per day and the rate of nicotine contained in cigarettes. Nevertheless, the variable such age and body mass index (BMI) don't influence the level of AA.

Conclusion: This study demonstrated that heavy smoking is associated with a decrease in serum AA levels.

Phthalates exposure and health implications

GT P–19

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Background & Aims: Phthalates are considered endocrine disruptors. They are present everywhere in our daily life and their widespread use promotes human exposure by various means. As a result, they are intensively researched to try to identify their potential health effects. The objective of this study is to estimate the daily exposure to phthalates in the general population.

Methods: This is a cross-sectional, analytical descriptive study conducted on a random sample of participants residing in the cities of Oran and Tlemcen over a one-year period to assess the rates of six phthalate metabolites (Mono

ethyl phthalate , mono-n-butyl phthalate, mono benzyl phthalate, mono-2-ethylhexyl phhtalate, mono-2- ethyl-5-hydroxyhexyl phthalate et mono-2-ethyl-5-oxohexyl phthalate) in urine samples. The determination of phthalates and their metabolites was carried out by GC-SM after processing our samples by liquidliquid extraction

Results & Discussion: The analysis of two hundred and fourteen urine samples revealed the presence of at least one phthalate in each sample with an interval of 18% to 100% with concentration range 29.60 µg/l -226µg/l. Mono butyl phthalate was present in the samples of all participants. The highest rates were noted in women with a significant difference (p=0.021) this difference may be due to a source of exposure that is the use of cosmetic products. The daily intake of phthalates exceeded the tolerable daily intake for DEHP (TDI:50µg/kg/j)and DBP (TDI:10µg/kg/j), in 6.5% and 13% of subjects respectively and 21% of the population exceeded the danger index(HI>1).

Conclusion: Improving the public's knowledge of the risk of phthalates is more than necessary to prevent its harmful effects on health throughout the life cycle

GT P-20

Participation of Greek cities in the ESCAPE (European Syringe Collection and Analysis Enterprise- EMCDDA) program for the year 2023 - entry of new cities and analysis of the results

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Background & Aims: The ESCAPE (European Syringe Collection and Analysis Enterprise) is a project of the European Organization EMCDDA (European Monitoring Center for Drugs and Drug Addiction) and Greece is part of the EMCDDA ESCAPE network since 2022. The object of ESCAPE is to detect psychoactive substances, old and new psychoactive substances (NPS) injected by drug users in a network of European cities and to monitor changes in usage patterns with time. In 2023, the Greek cities participating in the programme increased from two that participated in 2022 (Athens, Thessaloniki) to five. In 2023, used syringes were collected from different areas of Athens, Thessaloniki, Patras, island of Crete and Volos. The Greek Organisation Against Drugs (OKANA) was responsible for the collection of the syringes and the analysis was carried out at the Forensic and Toxicology Laboratory of the School of Medicine of the Aristotle University of Thessaloniki.

Methods: September has been set as the collection month for 2023 and a total of 1275 used syringes were collected and analyzed. A liquid chromatography-mass spectrometry system was used (Acquity H class, Waters coupled with 6500+ QTRAP, Sciex) and the results will be uploaded on the ESCAPE platform. Briefly, syringes were rinsed with 1 mL Methanol and the extracts were centrifuged (12000 rpm, 10min). A screening method that allows a wider range of substances to be detected (including new substances) was used. A required minimum list of substances has been established by the network, including classical drugs, new psychoactive substances (NPS), cutting agents, degradation products and metabolites are included in that list. The minimum set of substances is reviewed each year by the network.

Results & Discussion: From the preliminary analysis of the samples, statistical processing is still ongoing, it appears that in the cities where the syringes were collected,there is a trend in the simultaneous use of heroin and cocaine. Methamphetamine was mainly detected in syringes collected in the city of Athens. The detection of buprenorphine in the residual content of used syringes is of great interest and needs further investigation, as the same trend was observed in 2022 in certain areas of the city of Thessaloniki.

Conclusion: In the Greek cities that participated in 2023 in the ESCAPE program, the use of traditional intravenous drugs is mainly observed. This program records real-time psychoactive substances being used intravenously in the aforementioned cities and immediately informs policy makers about drug use and potential emerging health threats in the region.

GT P-21

Assessing food additives in carbonated drinks: Health implications and consumer awareness

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Background & Aims: The omnipresence of food additives in industrialized food indicates the need for research in the modalities of preparation and possible risks to health of consumers. The purpose of this study was to estimate food additives contained in carbonated drinks, which are very popular in the Algerian market

Methods: A survey was made on a sample of carbonated drinks marketed (n=50) in the region of Tlemcen over a period of 3 months to identify the content of food additives and to classify every additive as coloring agents, preservative, or regulator of acidityA survey was made on a sample of carbonated drinks marketed (n=50) in the region of Tlemcen over a period of 3 months to identify the content of food additives and to classify every additive as coloring agents, preservative, or regulator of acidity

Results & Discussion: The results revealed that eight categories of food additives are employed in the carbonated drinks studied: colorings agents, preservatives, antioxidants, emulsifiers and stabilizers, sweeteners, acidity regulators, carbonating and flavorings agents. Among the cited additives, regulators of acidity are the most used additives in the carbonated drinks studied; they are present in 98% of the samples, followed by preservatives (82%) and food colors (80%). A second study was made in parallel having an objective to evaluate the state of knowledge of the consumers on food additives used in carbonated drinks. It is a descriptive observational study, made on a random sample of 100 individuals, the results indicating that 66 % of the participants had no knowledge on food additives present in carbonated drinks, but 70 % were aware of dangers of carbonated drinks.

Conclusion: On the grounds of the present study it becomes clear that more efforts are needed to inform and to make aware the population of the risks for health to the presence of food additives in carbonated drinksOn the grounds of the present study it becomes clear that more efforts are needed to inform and to make aware the population of the risks for health to the presence of food additives in carbonated drinksOn the grounds of the present study it becomes clear that more efforts are needed to inform and to make aware the population of the risks for health to the presence of food additives in carbonated drinks

Phthalates in polyethylene terephthalate bottled water: Origins, migration factors, and health risk assessment

GT P-22

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Background & Aims: The presence of phthalates in PET bottled water has become a recent concern among some researchers, especially considering the steady growth of bottled water consumption and the toxicological effects of these substances. The objective of this study was to develop a systematic review to provide insight that encompasses the origins of phthalates in bottled drinking water as well as the factors that influence their migration.

Methods: A systematic literature search was conducted via Pubmed, Science direct and Google scholar. This work was conducted and written in accordance with Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA)guidelines

Results & Discussion: Phthalate contamination of drinking water stored in PET bottles was observed in all articles reviewed, which was assumed to be due to contamination of the water in the bottling plant; migration of phthalates from the bottle polymer into the water or by cross-contamination during analytical procedures in a laboratory. In addition to these origins, there are other factors that favor the migration of phthalates such as the improper storage of bottled water as well as their exposure to high temperatures or their conservation. In addition, we considered only the risk assessment associated with exposure to di-ethyl hexyl phthalate (DEHP) through the use of bottled drinking water and the majority of the results showed that regardless of the source of Phthalic Acid Esters PAEs in PET bottled water, the health risk associated with the selected levels of PAEs was acceptable and does not pose any significant concern to human health.

Conclusion: From our study it could be concluded that the adverse effects due to phthalates may be due to other sources of exposure in addition to bottled drinking water.

Intracellular uptake of hydrogen sulfide and stress granule formation in hydrogen sulfide-exposed human bronchial cells

GT P-23

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Background & Aims: Hydrogen sulfide (H2S) is a toxic gas with a characteristic rotten egg odor. Accidental acute exposure to high concentrations of H2S causes respiratory paralysis, unconsciousness, and eventually death within minutes. Stress granules (SGs) are phase-separated and membrane-less intracellular organelles composed of proteins and RNA. SGs are formed transiently and reversibly in response to various stressors such as arsenite, heat stress and osmotic stress, to protect cells from cytotoxic effects. In a previous report, we reported that H2S induced SG formation in human bronchial cells. In the present study, we have quantified intracellular H2S using H2S-specific fluorescent dyes and investigated effects of glutathione on SG formation in H2S-exposed cells.

Methods: CHO-K1 cells stably transfected with GFP-tagged Ras GTPase-activating protein-binding protein 1 (G3BP1) (G3BP1-CHO) and human bronchial epithelial cells BEAS-2B (BEAS) were used in the present study. Both cells were exposed to 0-10 mM NaHS (a donor of H2S) for 1 hour in a sealed culture plate. SG formation in G3BP1-CHO cells was observed as a live image of GFP-G3BP1 by fluorescence microscopy. BEAS cells were first fixed after exposure to NaHS, immunostained with anti-G3BP1 antibody, and SG formation was examined by fluorescence microscopy. Intracellular H2S in NaHS-exposed BEAS cells was detected and quantified using fluorescence probes for H2S, HSip-1 DA, and HSip-1 (Dojindo), respectively. BEAS cells were lysed with RIPA lysis buffer. Then phosphorylation levels of AMP-activated protein kinase (AMPK), as a cellular energy stress sensor, and eukaryotic translation initiation factor 2α (eIF2α), which is required for SG formation, were examined by Western blotting. In addition, the cells were exposed to NaHS in the presence of 0.1 mM buthionine sulfoximine (BSO), a glutathione depleting agent, or 5 mM dorsomorphin, an AMPK selective inhibitor, to study effects of these inhibitors on H2S-induced SGs formation and eIF2α phosphorylation.

Results & Discussion: The intracellular H2S was increased within 15 minutes of exposure to NaHS in a dose-dependent manner, indicating that H2S was rapidly incorporated into BEAS cells. G3BP1-CHO and BEAS cells formed discrete SG assemblies after exposure to 5 and 10 mM NaHS. In the presence of BSO, SGs were formed at a lower concentration (2.5 mM) than without BSO in both cell types. Phosphorylation levels of AMPK and eIF2α were significantly and dose-dependently increased by NaHS exposure in BEAS cells. In the presence of BSO, the phosphorylation level of eIF2α was significantly increased compared to that in the absence of BSO. The phosphorylation level of eIF2α was further enhanced by dorsomorphin in NaHS-exposed BEAS cells.

Conclusion: Our results suggest a possibility that H2S is rapidly taken up into the pulmonary cells and SGs are formed via phosphorylation of eIF2α to evade cytotoxic effects of H2S when humans are exposed to high concentrations of H2S. The formation of SGs was exacerbated by glutathione depletion, suggesting that glutathione, which facilitates H2S metabolism and ROS inactivation pathways, is indirectly involved in SG formation. Furthermore, inhibition of energy stress sensor enhanced eIF2α phosphorylation, possibly indicating that energy depletion by H2S might be related to the cellular stress responses, including SG formation.

GT P-24

Toxicokinetic study of amygdalin and cyanide in mice

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Background & Aims: Amygdalin is a natural compound found in plants such as almonds and apricots. Upon ingestion, amygdalin undergoes metabolism to cyanide, a toxic substance.

The aim of this study was to determine toxicokinetic parameters in mice following oral (PO) and intravenous (IV) administrations of amygdalin.

Methods: Toxicokinetic studies of amygdalin and cyanide, were conducted in mice after administration of IV doses (120 mg/kg) and PO doses (150 mg/kg) of amygdalin. Amygdalin levels were measured at T 10, 30, 60, and 90 minutes, while cyanide levels were measured at 10, 30, 60, 90, 120, and 180 minutes. Amygdalin levels were measured using HPLC-UV with a C18 column (250 mm x 4.6 mm, 5 µm), employing a mobile phase consisting of 60% methanol and 40% water. Detection was performed at 215 nm, with a total analysis time of 13 minutes. Cyanide levels were measured using LC-MS/MS with a C18 column (150 x 2.1 mm, 5 µm), employing a mobile phase of 2 mM formate buffer in 0.1% formic acid and acetonitrile. Cyanide ions detected were m/z 299.0, with product ions of 81.2 and 191.1, and a total analysis time of 5 minutes. The study has been approved by the ethics committee.

Results & Discussion: The AUC0-inf value of amygdalin obtained after IV administration was 13997.12 (mg/L)* min. The half-life (t1/2) was 31.06 min. The apparent volume of distribution (Vss) was 0.41 L/kg and the clearance (CIT) was 0.009 kg* L/min.

Amygdalin was not detected in plasma after oral administration in this study. Studies have shown that orally taken amygdalin is not effectively absorbed but is partially converted to prunasin and released as cyanide in the cecum.

After oral administration of 150 mg/kg of amygdalin in mice, the maximum (Tmax) of cyanide was 120 min. The AUC0-inf (ug/L*min) value obtained after PO administration was 196.49 mg/L* min. The half-life (t1/2) was 43.82 min.

Conclusion: These findings highlight the complex metabolic fate of orally ingested Amygdalin. Further studies are needed to fully understand the metabolism and toxicity of this compound.

Regulatory status of methanol in surgical alcohol: Implications for counterfeit alcohol in Algeria

GT P-25

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Background & Aims: In Algeria, surgical alcohol has a dual regulatory status: cosmetic and health product. The objective of this work is to present the regulatory status of surgical alcohol in Algeria, particularly the authorized methanol levels and associated health risks.

Methods: In December 2023, 14 people died after consuming counterfeit alcohol in Algeria, which reportedly contained a mixture of surgical alcohol and another substance according to the press. Subsequently, within the scope of this study, regulatory texts from the Ministry of Industry and Pharmaceutical Production and the Ministry of Trade and Export Promotion were examined.

Results & Discussion: In Algeria, ethyl alcohol is classified as a pharmaceutical product as it is listed in the national classification of pharmaceutical products for human medicine. It is subject to a limit of 200 parts per million (ppm) for methanol in alcohol. Ethyl alcohol may also be used in the formulation of cosmetic and personal hygiene products. In this case, methanol compared to ethanol or propanol-2 has an acceptable limit of Maximum 5% calculated as a percentage of ethyl and isopropyl alcohols.

Conclusion: Understanding the regulatory framework surrounding surgical alcohol in Algeria, including permissible methanol levels, is crucial for mitigating health risks associated with counterfeit alcohol consumption and ensuring public safety.

Trends of other drugs detected in positive gamma-hydroxybutyric acid (GHB) from 2019 to 2023

GT P-26

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Background & Aims: Sodium oxybate, commonly known as gamma-hydroxybutyric acid (GHB), is an approved medication for narcolepsy treatment but is also illicitly produced and abused for its sedative and euphoric effects. GHB can be found both exogenously and endogenously. In illicit use, GHB is referred to by various street names such as Georgia Home Boy, Grievous Bodily Harm, Easy Lay, Liquid Ecstasy, and Liquid X. The effects of GHB occur in as little as 15-30 minutes and may last for three to six hours. GHB is known to increase the CNS depressant effects of alcohol and vice versa. GHB may also cause amnesia, increase libido, and anesthetic effects which have resulted in those who consume GHB unknowingly to be susceptible to criminal acts such as sexual assault. GHB consumption may lead to seizures, unconsciousness, coma, or death. This study aims to analyze trends in co-occurrence of GHB with other drugs in postmortem, antemortem, and drug-facilitated crime panel (DFCP) cases.

Methods: Cases over a five (5) year period (2019-2023) that underwent routine forensic testing procedures were reviewed for this investigation. The review focused exclusively on cases with positive GHB findings to determine the co-occurrence of other drugs. Over the five-year period, there were 738 cases where GHB was detected. Of the 738 cases, there were 267 cases positive for GHB and additional substances. GHB is quantified using gas chromatography/mass spectrometry (GC/MS) with an analytical method range of 5.0 mcg/mL – 200 mcg/mL. This study examines the positive GHB cases with other drugs measured in the specimens received.

Results & Discussion: Positive GHB cases with other drugs detected ranged from 25 in 2019 to 71 in 2022 and 70 in 2023 and included blood, serum/plasma, and urine matrices. Each year exhibited an increase in unique drugs co-occurring with GHB positive cases. In 2019, 41 different drugs were detected alongside GHB that increased to 93 in 2023. Co-occurring drugs were categorized into nine groups: CNS depressants, CNS stimulants, hallucinogens, dissociative anesthetics, narcotic analgesics, inhalants, cannabis, miscellaneous, and incidental findings. Excluding miscellaneous and incidental findings, the percentage distribution of the seven main groups varied across the years. Also, there were no inhalants detected in any of the samples reviewed. The data for these different groups of drugs are displayed in the table below.

Table 1: Prevalence of Types of Co-occurring Drugs with Positive GHB cases from 2019-2023.

	CNS Depressants	CNS Stimulants	Hallucinogens	Dissociative Anesthetics	Narcotic Analgesics	Cannabis
2019	27.9%	46.5%	1.16%	1.16%	16.3%	6.97%
2020	29.1%	44.1%	1.57%	3.14%	13.3%	8.66%
2021	26.1%	33.6%	4.20%	0%	25.2%	10.9%
2022	35.3%	28.9%	1.83%	0.45%	16.5%	17.0%
2023	35.7%	31.5%	1.40%	0.46%	21.1%	9.85%

Overall, the trends observed among the occurrence of positive GHB samples with CNS stimulants exhibits a decrease over the five-year study starting with 46.5% in 2019 and decreasing to 31.5% in 2023. There was also a small increase of CNS depressants and narcotic analgesics detected with positive GHB cases. In 2019 and 2020, common co-occurring drugs included amphetamine, methamphetamine, ethanol, and cannabis, while 2021 showed a shift up in the use of fentanyl alongside these substances. In 2022 and 2023, ethanol, cannabis, amphetamine, methamphetamine, and fentanyl predominated. Notable findings over the five-year study also included cocaine, benzoylecgonine, para-fluorofentanyl, metonitazene, protonitazene, nitrates, alpha-hydroxyetizolam, flualprazolam, and flubromazolam. Some known limitations of positive GHB samples include a relatively short window of detection due to a short half-life of GHB, endogenous production, and in vitro formation while cases are stored.

Conclusion: The frequency of positive GHB cases with other drugs detected increased almost three-fold over the five-year period in the cases studied. During this time frame, the main drugs present with GHB positive cases has remained relatively unchanged; however, there has been a notable increase in the variety of co-occurring drugs. Continuous monitoring of drug trends and prevalence is essential for forensic toxicology laboratories to effectively analyze submitted casework.

GT P-27

Occurrence and fate of selected heavy metals and their removals in a wastewatertreatment plant

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Background & Aims: Since the mid-20th century, a mounting body of scientific evidence has emphasized harmful effects of heavy metals on both human health and the delicate equilibrium of aquatic ecosystems. These metals pose significant risks due to their characteristic toxicity and tendency to bioaccumulate within soil, water and consequently, food chains.

Agricultural (fertilizer, pesticides applications, burning of fossil fuels) and household (e-wastes, car washes, dental uses, transport-related emissions) sources are severe heavy metal contributors to the sewage system beside industrial activities Though, in recent years, worthy steps have been taken in reducing emissions from metal production facilities across many nations. Even with these improvements, however, worries continue about the spread of toxic elements from using and throwing away everyday products containing heavy metals.

The aim of this research was to investigate the accumulation and removal of heavy metals (Li, B, Mg, Al, P, Ca, Cr, Mn, Co, Ni, As, Se, Sr, Cd, Sn, Sb, Ba, Hg, Cu, Pb, Zn) using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) in a biological wastewater treatment system. Additionally, to compare heavy metal concentrations in the influent and effluent wastewater, and further evaluate whether effluent heavy metal levels exceed the desirable limits set by the World Health Organization (WHO) standards.

Methods: Composite 24-h wastewater samples both influent and effluent were collected for 7 consecutive days and sampling was carried out at 3-month intervals to monitor temporal changes (September and December of 2023 and March of 2024). A total of 42 samples were collected to high density polypropylene containers as time-proportionally and transferred to the laboratory. The samples were tested for biological oxygen demand (BOD), chemical oxygen demand (COD), pH, temperature, total nitrogen, and total phosphorus in the catchment plant and results recorded.

After adding 1 mL of 65% HNO3 to 1 mL of homogenized wastewater in a PTFE digestion vessel, the closed vessels were placed in a microwave oven set to 90 °C for one hour (peak power: 1600 W, pressure: 500 psi) for digestion. The digests were cooled, then placed into washed volumetric flasks, diluted ten times with ultra-pure water, and mixed with the internal standard (IS) solution mixture prior to ICP-MS analysis. The calibration solutions were prepared daily in 2% HNO3 by incremental amount as in between 0.01-100 ng/mL except Sn. All calibration solutions and samples were added IS (20 ng mL-1 of Ga and In) and were prepared in triplicate. The blank samples were digested and processed without wastewater in order to determine the possible contaminations or interferences.

The concentration differences between influent and effluent wastewater samples were evaluated proportionally against effluent samples to determine how much of the elements were eliminated as a result of the treatment processes. The student's t-test was performed between influent and effluent results. The temporal changes in heavy metal concentrations and possible limit exceedances in effluent samples were investigated by using Microsoft Office Excel 2021 (version 16.0.14332.20649).

Results & Discussion: Flow rates were between 12774-13498 m3/day, the mean BOD and COD values were 12.33 and 21.66 µg/L for three sampling campaigns, respectively. All of the targeted elements were found to be above the detection limits in both influent and effluent samples. The results were presented as mean values of 7-day sampling for each campaign. According to the results of the study, heavy metal loads in the influent samples of September 2023 were found to be in the 0.18 to 105285.71 µg/L range, while heavy metal loads in the effluent were found to be in the 0.09–44153.33 µg/L range. As for December 2023 sampling, influent sample loads were in between 0.25-97438.57 µg/L, while effluent loads were 0.10-45031.43 µg/L. Lastly, March 2024 results ranged between 0.23 to 118674.29 µg/L for influents and 0.02 to 64982.86 µg/L for effluent samples. For every campaign, Mg and Ca had the highest concentrations while Cd and Hg had the lowest in both types of wastewater samples.

According to the Student's t-test results of each period, the removal of Li, B, Al, P, Ca, Mn, Ni, Cu, As, Sr, Cd, Sb, Ba, Pb were statistically significant ($p<0.05$). None of the heavy metal concentrations found in the effluents were found to be above the acceptable limits set by WHO.

Conclusion: Many countries have taken commendable action in the last few years to reduce emissions from metal producing facilities. This progress can be attributed to a combination of regulatory measures, technological advancements, and shifts in industrial practices aimed at minimizing environmental impact. The results of the analysis of a single facility in this study suggest that the efforts have been successful, but more comprehensive studies should be carried out at regular intervals across the country to address the issue comprehensively. In essence, the ongoing battle against heavy metal contamination necessitates a concerted effort from scientific, regulatory, and societal stakeholders.

Increase of pregabalin misuse among forensic toxicological analysis in Belgium

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Background & Aims: Pregabalin is a drug indicated for the treatment of epilepsy, neuropathic pain and generalised anxiety disorders. Since 2016, pregabalin has also been known to be associated with risks of abuse, misuse and pharmacodependence. The scientific literature shows that the misuse of pregabalin is increasing among opiate addicts: diversification of the methods of administration (intranasal or intravenous) have been reported. The aim of this study was to document the recreational use of pregabalin using toxicological expertise data from Belgian forensic cases distinguishing the different type of cases and to give an interpretation to the Belgian judicial district.

Methods: A retrospective analysis was performed concerning the detection of pregabalin in the biological fluids from living or deceased persons, collected by our laboratory in the context of Belgian forensic cases between 2020 and 2023. The method used for the detection of pregabalin was the same throughout the study period. Driving Under the Influence of Drugs (DUID) cases were excluded because the analytical strategy for these files is different. Pregabalin is detected by UPLC-QTOF in cases where a comprehensive drug screening is required such as in drug-facilitated sexual assaults, death, murder, narcotics, theft or aggression cases.

GT P-28

Results & Discussion: Over the study period, the number of cases in which pregabalin was detected increased, in all type of forensic analyses, whether in cases of misuse, postmortem analysis or general toxicology. Although the casework increased from 280 to 420 cases over the study period, the pregabalin detection rate fell from 1% (n =3) to 5% (n=19). On the 19 cases highlighted for 2023, pregabalin was mostly combined with benzodiazepines (n = 18, 95%), opioids (n = 12, 63%), cocaine (n = 12, 63%) or antidepressants (n = 7, 37%). Blood concentrations measured ranged from 0.1 to 5.8 µg/ml (median: 1.65 µg/ml; mean: 2.2 µg/ml). Data from the National Institute for Health and disability Insurance (NIHDI) also show a significant increase in the number of patients receiving pregabalin on prescription, rising from 5 318 in 2006 to 165 485 in 2022.

Conclusion: The number of forensic cases detecting pregabalin increased between 2020 and 2023. These results should be related to the increase of pregabalin misuse reported by the inspection services of the Federal Agency for Medicines and Health Products (AFMPS) and by the National Institute for Health and disability Insurance (NIHDI). Findings from the present retrospective study have implications for identifying subgroups who could benefit from prevention and critical points for intervention.

GT P-29

Comparison of characteristics of serious chemical accidents before and after enforcement of the Serious Accidents Punishment Act in South Korea: NFS case-based accident substances

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Background & Aims: The Serious Accidents Punishment Act (Act no. 17907, enacted on January 26, 2021, implemented on January 27, 2022) in South Korea is a law implemented with the purpose of preventing serious disasters by strengthening the level of criminal punishment for business owners, public officials, and corporations that cause casualties by neglecting safety and health measures. The term `serious accident' means a `serious industrial accident' or a `serious civic accident' and is defined as an accident in which two or more people are seriously injured or at least one person has died. In this study, we compared the characteristics of serious chemical accidents by reviewing chemical accident cases submitted to the National Forensic Service (NFS) before and after the enforcement to evaluate the impact of the law's implementation.

Methods: A total of 58 cases from 2011 to 2018, the period before the enforcement of the law, and 28 cases from 2022 to 2023, the period after the enforcement of the law, were reviewed. The 2020-2021 COVID-19 pandemic period was excluded from the review due to low number of cases.

Results & Discussion: From 2011 to 2018, the frequency of accident-causing substances was oxygen deficiency (21%), carbon monoxide (19%), hydrogen sulfide (14%), organ ic solvents (12%), combustible gases (9%), strong acids (7%), freon refrigerants (4%) and others (14%). On the other hand, during 2022-2023, the frequency was organic solvents (29%), carbon monoxide (21%), hydrogen sulfide (21%), freon refrigerants (11%), combustible gases (7%), oxygen deficiency (4%) and others (7%).

When comparing before and after the implementation of the new Act, oxygen deficiency accidents, which accounted for the largest proportion of 21% in the period 2011-2018, decreased significantly to 4% in 2022-2023. It is also noteworthy that strong acid accidents completely decreased from 7% to 0% during the same comparison period. Meanwhile, even after the enforcement of the Act, carbon monoxide and hydrogen sulfide accidents were still ranked as the top three causes of accidents, and in particular, the frequency of organic solvent accidents increased noticeably compared to the period before enforcement.

Oxygen deficiency accidents have mainly occurred in small-scale workplaces such as manholes and garbage disposal sites, and the main causes of the accidents were human factors such as lack of sufficient ventilation before work and non-attachment of oxygen sensors. It is noteworthy that oxygen deficiency accidents, which were the number one cause of serious accidents showed the greatest decline since the enforcement of the punishment act. And the complete reduction in serious accidents caused by corrosive acid chemicals after the enforcement of the act is expected to be the result of strengthening preventive measures following major national disasters such as the hydrogen fluoride leak at the plant in 2012 and 2013 and the sulfuric acid leak at the plant in 2016. On the other hand, organic solvent accidents have accounted for the largest proportion in the past two years while the punishment act was in effect. They were mainly fires, explosions, and poisoning accidents during painting or cleaning work, and more strengthened safety management is needed.

Conclusion: Comparison of characteristics of serious chemical accidents before and after the implementation of the new act may not directly indicate the effectiveness of changes in the legal environment(new laws and regu-

lations). The limitations of this study are that there are many variables that affect the occurrence of accidents other than the new law, and that it covers a limited number of cases. We acknowledge that the effectiveness of new regulations and systems should be evaluated from various perspectives with a large number of accidents and cases. Nevertheless, as an initial report on changes in serious chemical accidents after the implementation of the Serious Accident Punishment Act, this study can be a way to evaluate the impact of new Act.

Poster gallery - HR F-P-1 to P-11

16:00 - 16:30 Thursday, 5th September, 2024

A routine and metabolomic-based LC-HRMS method developed for the indirect detection of dopaminergic manipulation in equine urine.

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Background & Aims: Current accepted methods of urinary 3-methoxytyramine (3-MT) analysis for the indirect detection of dopaminergic manipulation in the equine athlete industry are based on gas chromatography – mass spectrometry (GC-MS) and utilise a time-consuming sample preparation method featuring sample hydrolysis, solid-phase extraction and derivatisation components. This study aims to develop a new analytical method to improve sample preparation efficiency and method sensitivity by adapting a liquid chromatography – high resolution mass spectrometry (LC-HRMS) method developed by Keen et al. (J Chroma B, 2023) for biomarkers in equine plasma. The adaption of this method was conducted for both quantitative (3-MT, with the addition of tyramine) and qualitative (phase I and II metabolite feature extraction) purposes, and "field-tested" using a pilot study involving the administration of dopaminergic Parkinson's disease medication, Stalevo 100 (25 mg carbidopa, 100 mg levodopa, 200 mg entacapone), in mares and geldings.

Methods: Urine (1 mL) was combined with an acetate buffer solution (1 mL) and enzyme (β-glucuronidase, 50 µL), pH adjusted to 5 – 5.5 and hydrolysed at 37 °C for 12 hours (overnight). The urine and buffer mix solution was aliquoted (200 µL) with acetonitrile (ACN, 300 µL) and internal standard (10 µL, 4 µg/mL equivalent of 3-MT d4) in a microcentrifuge tube (600 µL capacity). The solution was capped and vortexed before being micro-centrifuged at 18000 x g for 5 minutes. The supernatant was then transferred to small glass test tubes with methanolic hydrochloric acid (20 µL), before drying under nitrogen at 60 °C. Reconstitution involved 20 mM ammonium formate solution in 80% ACN (200 µL). A routine C18 column guard was used in conjunction with an Imtakt Intrada amino acid analytical LC column (2.1 x 100 mm, 3 µm), and samples analysed using electrospray ionisation, positive mode on the Shimadzu LC-QTOF-MS 9030 instrument. Mobile phases used were A: 0.3% formic acid in 100% ACN, and B: 100 mM ammonium formate in 100% water.

An adaptation of this untargeted method was optimised further to remove the need for hydrolysis and started by aliquoting a reduced volume of urine sample (100 µL) with ACN (300 µL) and internal standard (10 µL, 200 ng/mL equivalent of an IS mix) in a microcentrifuge tube (600 µL capacity). This metabolomic approach captured data using a data independent acquisition (DIA) method, and results were normalised and aligned in MS-Dial, before feature extraction/statistical processing in MetaboAnalyst 6.0.

Results & Discussion: The adapted Keen et al. method was further optimised for a urine matrix analysis, involving; the protein precipitation solvent type, addition of hydrolysis for conjugated compounds to follow current routine processes, and the reduction in column flowrate from 0.5 mL/min to 0.3 mL/min to account for analyte retention on the column and optimal peak shape. The quantitation of 3-MT and tyramine was conducted using a 2 – 8 µg/mL calibration range, spiked in a non-hydrolysed urine matrix. This range sits in line with the current urinary detection threshold for 3-MT in equine samples at 4 µg/mL. A metabolomic feature extraction of free and intact conjugated metabolites was analysed in both positive and negative mode to obtain other potential biomarkers for further investigation. Additionally, the identification of potential biomarkers may be validated with use of a reference population study in future to test their robustness. Results from a small pilot study analysis show promising results for complementary use with current routine GC-MS processes in an equine racing context.

Conclusion: A fit-for-purpose LC-HRMS method has been developed and validated for the indirect detection of dopamine manipulation in equine urine via 3-MT and tyramine. Additionally, the use of a metabolomic search for other potential conjugated biomarkers, currently unknown, shows promise for improvements in current detection methods and timeframes. This method suits a complementary GC-MS and LC-MS analysis approach within a routine anti-doping laboratory.

HR F-P-02

Application of an untargeted metabolome approach to screen for biomarkers of nitrite poisoning

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Background & Aims: Sodium nitrite is an inorganic compound commonly used as a preservative in the fish and meat industry. You can even find instructions on how to use sodium nitrite for suicide online. Ingestion of higher doses of nitrite is indeed associated with high toxicity in humans because nitrite can oxidize hemoglobin to methemoglobin, leading to severe tissue hypoxia and death. Despite the continuous optimization of analytical toxicology, analytical (direct) detection of nitrites generally remains challenging, and other clues (findings at the crime scene or autopsy, e.g., brownish blood due to methemoglobinemia) are usually required to consider ingestion or overdose of nitrites in a given case. Therefore, we aimed to search for potential (indirect) endogenous biomarkers of nitrite exposure in whole blood samples that could ideally be included in routine screening approaches.

Methods: Whole blood samples collected from seven healthy volunteers were pooled and incubated in vitro without and with different concentrations of sodium nitrite (1mM, 5mM and 10mM, n=10 each) under agitation at 35°C for 1 h. Subsequently, samples were extracted by adding 500 µL methanol:acetone (90:10 v/v) and stored overnight at -20°C to ensure protein precipitation. The samples were then centrifuged and analyzed using a non-targeted liquid chromatography-high resolution mass spectrometry (LC-HRMS; Waters HSST3 column, Sciex X500R, ESI+) method. A metabolome-like approach was applied for data evaluation, using MS-DIAL 4.9 for peak selection, followed by an unpaired statistical analysis (Mann-Whitney test, ANOVA, $p<0.05$) to filter out the different features between the control and nitrite samples and between the different concentrations. Feature identification was attempted using the Sirius software with commercially available databases (COCONUT, HMDB, NORMAN, PubChem, PubMed, YMDB, etc.).

Results & Discussion: Selection of the appropriate NaNO2 concentration for in vitro fortification was done based on current literature. 1mM and 5mM were chosen as they are reported in authentic deaths by nitrite, while 10mM were used to simulate an enhanced phenomenon and interpret the results. Following data processing, 176 total features were found to be significantly different (fold change >1.5 or <0.66 and p-value < 0.05) between the control and nitrite-treated samples. Of the 176 total features, 25 were identified with a library match greater than 65%, and 20 of them with a match greater than 80%. Additionally, intensity of 10 out of 25 (40%) features decreased with increasing concentration, while the remaining 60% showed an increase in expression with increasing concentration. Features included indole-3-carboxaldehyde, formylanthranilic acid, indoleacetic acid, three compounds involved in tryptophan metabolism, uric acid, glutathionate and compounds belonging to the class of lipids and glycerophospholipids.

Conclusion: A comprehensive workflow to investigate the metabolic profile of NaNO2-treated blood is presented. Several interesting biomarkers were identified, but the concentrations studied and the results reported so far, although promising, are based on in vitro studies only; further studies, including in vivo case samples, will be needed in the future.

HR P-03

T-ReXing NPS Data – Unveiling metabolite features suitable for urine screening via UHPLC-timsTOF-MS

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Background & Aims: Urine one of the preferred matrices for standard toxicological analysis, making the implementation of drug metabolites in libraries or databases crucial for targeted and untargeted mass spectral screening approaches. Unfortunately, the availability of reference standards for drug metabolites, particularly for New Psychoactive Substances (NPS) like synthetic cannabinoid receptor agonists (SCRAs), is limited. As the parent substance for SCRAs itself is rarely detectable in urine, the elucidation of the metabolism is a major challenge for forensic toxicology laboratories. To be able to detect the use of new SCRAs, biomarkers for consumption must be swiftly integrated into screening methods post-seizure. Pooled human liver microsome (pHLM) assays are an easy-to-use and cost-effective methodology to generate phase I metabolites when the parent compound is available. Trapped ion mobility spectrometry (TIMS) high resolution MS combined with sophisticated software tools can be used to predict and annotate features of potential metabolites.

Quetiapine, a neuroleptic yielding multiple well known metabolites, was used as model substance to demonstrate the suitability of a non-targeted workflow using UPLC-timsTOF-MS and MetaboScape, incorporating in silico pre-

diction of metabolites, as well as collision cross section (CCS) prediction and in silico generation of MS fragments. Subsequently, this workflow was used to elucidate metabolic features of three highly prevalent SCRAs.

Methods: Potential quetiapine and SCRA phase I metabolites were generated in vitro using an established pHLM assay. The highly prevalent SCRAs ADB-BUTINACA (ADB-BINACA), MDMB-4en-PINACA and the newly emerged MDMB-BUTINACA (MDMB-BINACA) were selected as representatives of this NPS subset. Analysis was performed using an Elute UHPLC connected to a Bruker timsTOF Pro 2 equipped with a VIP-HESI ion source operated in positive mode. Metabolite prediction was performed using Biotransformer 3.0. Features were extracted in MetaboScape using the T-ReX 4D algorithm (Time aligned Region complete eXtraction) and annotated using CCS prediction and in silico fragmentation of the predicted drug metabolites. Known quetiapine metabolites were confirmed using HR spectral data from the MMHW library (Wiley-VCH). Forensic samples with known quetiapine or respective SCRA uptake were re-acquired in positive PASEF (parallel accumulation serial fragmentation) and bbCID mode to identify major in vivo metabolites. Retention time, CCS values and qualifier ions found in this step were added to the TargetScreener 4D database.

Results & Discussion: The metabolic pathway of quetiapine includes sulfoxidation, N-dealkylation, hydroxylation and carboxylic acid formation on the ethoxyethanol sidechain. The described workflow led to the annotation of 17 phase I metabolites in the pHLM assay. Twelve metabolites could be confirmed by library match with the MMHW library. Among those, the most abundant metabolites included mono-hydroxylated species, N- and O-dealkylated species. The top three most abundant biotransformation products in human urine samples were a monohydroxy metabolite, along with N- and O-dealkyl metabolites, followed by compounds exhibiting both hydroxylation and dealkylation.

For ADB-BUTINACA several monohydroxylations and deamination at the linked group were predicted and could be successfully annotated as well as for an ester hydrolysis metabolite at the methoxy moiety for MDMB-4en-PINACA, a relatively nonspecific but high abundant metabolite in vivo. The combination of ester hydrolysis combined with monohydroxylation or dihydrodiole formation at the Npentenyl side chain of MDMB-4en-PINACA, respectively, could not be predicted in silico. Nevertheless, these biotransformation steps could be predicted and subsequently annotated using the ester hydrolysis metabolite as the target compound for metabolite prediction. In addition, at least two monohydroxylated metabolites could be annotated in the majority of urine samples. Inter-individual differences in enzyme activity or different time periods between uptake and urine sampling might be an explanation for this.

For the novel SCRA MDMB-BUTINACA, monohydroxylated compound specific biomarkers could be predicted and successfully annotated in pHLM extracts and urine samples. Similar to MDMB-4en-PINACA, a hydrolysis metabolite as well as the combination of hydrolysis and hydroxylation were predicted and successfully annotated. This hydrolysis metabolite is also formed after the ingestion of ADB-BUTINACA but to a much lesser extent.

Conclusion: Qualitative findings from pooled human liver microsomes (pHLM) and urine samples were largely congruent. MetaboScape was able to detect and annotate high abundant and compound specific metabolites. Predicted CCS values and fragments helped to gain confidence in the annotation. However, it must be noted that the in silico prediction of metabolites is the bottleneck of this approach. Some major SCRA metabolites were not predicted in silico or required further manual processing steps to predict them. This might become especially challenging with the occurrence of SCRAs with uncommon substituents such as 3-trimethylsilylpropyl sidechains like ADMB- and Cumyl-3TMS-PrINACA.

This new workflow provides a sensitive detection as well as confident annotation and identification of multiple drugs and their metabolites. Due to the successful combination of in silico prediction and data from pHLM or real samples, respectively, reference standards of metabolites are no longer absolutely necessary - if available at all - to add their features to an MS screening to detect new emerging NPS in urine.

Ensuring consumer health: Diagnostic ion screening for illicit substances and prescription drug detection in gas station supplements

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HR P-04

Background & Aims: Dietary supplements (DSs) encompass a wide range of products, including vitamins, minerals, botanicals, amino acids, and other substances, intended to supplement the diet. In a study conducted by Mishra et al., 2023, from 2017 to March 2020 the usage of DSs among children, adolescents, and adults in the United States was examined. Findings revealed that approximately one-third of children and adolescents used DSs in the past 30 days, with higher usage among females. Among adults, 58.5% reported DS use, with higher usage among fema-

les across various demographic groups. While many individuals consume dietary supplements for various health benefits, concerns regarding their safety and regulation have been raised. Ensuring the quality and safety of dietary supplements is a significant challenge due to the sheer number and diversity of products available.

In this study, a collection of dietary supplements was purchased from gas stations and convenience stores and screened for a range of substances, including novel psychoactive substances, prescription medication, and a variety of botanicals. The samples were chosen based on their popularity and were screened against MS/MS spectral libraries. Several nondisclosed compounds were identified, including prescription medication. Diagnostic ion screening was then performed using Molecule Profiler to identify any impurities related to these prescription drugs.

Methods: Fourteen of the most popular dietary supplements were purchased from gas stations and convenience stores. The supplements were homogenized before analysis and 1 g of sample was placed into 10 mL of acetonitrile. The samples were then vortexed for 1 minute before centrifugation for 5 minutes. Following this, the samples were passed through a 0.22 µm PTFE syringe filter and diluted 1:100 using acetonitrile. Some samples did require additional dilution to maintain acceptable peak shape.

Liquid chromatography was performed using a Shimadzu LC-40 at a flowrate of 0.6 mL/min using a Phenomenex Luna Omega 3 µm Polar C18, 100 x 2.1 mm (Phenomenex Torrance, CA). The injected sample volume was 10 µL. Mobile phases A and B were Optima grade water with 0.1% formic acid and 5 mmol of ammonium formate and Optima grade acetonitrile with 0.1% formic acid, respectively. Mobile phase B was ramped using a linear gradient from 5 to 100% between 3 and 15 minutes. Mobile phase B was then held for 5 minutes at 100%. Samples were then injected into the ZenTOF 7600 system. MS/MS fragmentation was acquired using SWATH DIA analysis using both collision induced dissociation (CID) and electron activated dissociation (EAD) fragmentation methods.

Results & Discussion: Seven of the 14 samples disclosed having and did contain Kratom (Mitragyna speciosa). One of the most popular supplements contained a compound called tianeptine, which was not disclosed as an ingredient. In addition to a library fit score of 100 and a parent mass error of -0.5 ppm, an analytical standard was purchased allowing for a level 1 confirmation. In addition to these and other library matches, one of the other supplements contained prescription medications Tadalafil and Sildenafil. Diagnostic ion screening was then used, and 18 impurities related to sildenafil were identified. Several of these compounds were found in spectral libraries, however, many were not found in the literature.

Conclusion: Using high resolution mass spectrometry, several compounds that were not disclosed as ingredients in the supplements were discovered. Notably, the presence of tianeptine and prescription medications, like Tadalafil and Sildenafil, raise concerns about the accuracy of labeling and potential health risks associated with these undisclosed substances. The use of diagnostic ion screening allows for a more comprehensive characterization of these samples through the prioritization of potential compounds of concern even when they are not present in spectral libraries.

HR P-05

Simultaneous screening and quantitation of drugs and their metabolites in post-mortem vitreous humor by liquid chromatography-high resolution mass spectrometry

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Background & Aims: The utility of vitreous humor as an alternative matrix for post-mortem toxicological analysis has been limited by sample volume and the availability of suitable analytical methodologies. The advent of combined screening and quantitative methodologies using liquid chromatography-high resolution mass spectrometry (LC-HRMS) makes it possible to use low sample volumes to perform wide-ranging toxicological analysis. Vitreous humor is less prone to post-mortem redistribution, the effects of trauma, and has longer sample stability. It is thought to be closer to blood in comparison to other tissue types, with many compounds present in blood also found in vitreous humor. Therefore, a method for screening vitreous humor and measuring toxicologically significant compounds is likely to be useful in cases where no blood is available, or where the blood is subject to trauma or post-mortem redistribution. The aim of this study is to investigate and validate an existing combined screening and quantitative methodology to determine if it is suitable for vitreous humor.

Methods: 7-point calibrators for 51 toxicologically significant drugs and metabolites were prepared in whole blood and bovine vitreous humor and analysed in accordance with the methodology published by Rab et al.1 Each set of calibrators was analysed alongside low, therapeutic and high range standard solutions in vitreous humor (5 x 3 replicates) and inter and intra assay %CV and % bias calculated. Results generated using whole blood and vitreous humor calibrators were compared. Triplicate analysis of standard solutions in vitreous humor subjected to doubling dilutions was used to calculate limit of detection (LOD) and limit of quantitation (LOQ). A comparison of responses from 10 blank vitreous humor samples with responses from blank samples analysed following a

high standard was used to assess carryover. An assessment of ion suppression/enhancement was performed with post-mortem vitreous humor samples, including some containing fluoride oxalate preservative, and a search was performed for potential interferences from isobaric compounds. Whole blood calibrator solutions analysed on 5 separate batches were used to define the linear range over which R2 was > 0.997. Dilutional integrity was assessed by dilution of high concentration standard solutions (10 mg/L and 1 mg/L) 1:2, 1:5 and 1:10 with bovine vitreous humor. Post-mortem blood, urine and vitreous humor samples were screened using the Tox ExplorerTM library of compounds (Thermo Scientific, Waltham MA, USA) and results compared.

Results & Discussion: Precision and bias data showed that the use of whole blood calibrators gave acceptable quantitative results in vitreous humor samples. Whilst the use of matrix-matched calibrators is preferable in post-mortem analysis, the results showed that whole blood calibrators can be used where vitreous humor is not readily available. Intra and inter assay %CV precision ranges for 51 compounds were established for low (1.35 to 15.19, 3.68 to 17.03 respectively), therapeutic (3.07 to 16.00, 4.35 to 17.12) and high (2.04 to 8.29, 4.87 to 16.55) range standard solutions. % bias for each compound verses the expected concentrations in low, therapeutic, and high concentration standard solutions ranged from -15.72 to 25.03, -14.32 to 19.14, and -19.90 to 19.78 respectively. LOD was at least 0.05 mg/L, with most compounds significantly lower, and LOQ at least 0.1 mg/L, except for paracetamol which had a LOQ of 0.5 mg/L. There was no significant carryover, ion suppression or interference from isobaric compounds. The linear range for each compound allows for measurement of therapeutic and toxic concentrations. Quantitative results were within ± 20% of the expected target value when standard solutions (10 mg/L and 1 mg/L) were diluted 1:2, 1:5 and 1:10 with bovine vitreous humor, showing acceptable dilutional integrity. A comparison of post-mortem screening results obtained in blood and urine with results obtained in plain and preserved vitreous humor demonstrated that most compounds were detected in all matrices.

Conclusion: An existing combined screening and quantitative approach using LC-HRMS for post-mortem blood and urine is suitable for use with vitreous humor. A full validation of the methodology for 51 compounds in vitreous humor was performed and showed acceptable performance. The use of whole blood calibrators to quantitate compounds in vitreous humor allows for the use of a single set of calibrators in a batch measuring compounds in both blood and vitreous humor. Comparison of screening results between blood and/or urine and vitreous humor revealed that most compounds could be detected in all matrices.

References

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Identification of benzodiazepines in biosamples by determination of their comprehensive and characteristic metabolite profiles using LC-HRAM-Orbitrap-MS analysis

HR P-06

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Background & Aims: Benzodiazepines (BDZs) are among the most prescribed, used, and abused psychoactive substances worldwide. It is therefore important to develop analytical tools capable of identifying with certainty the possible presence of specific BDZs and their metabolites in biosamples, even in the absence of analytical reference standards. Analytical specificity can be greatly enhanced by identifying and applying comprehensive and characteristic metabolite profiles of each individual BDZ.

The study describes the application of liquid chromatography high-resolution accurate-mass Orbitrap mass spectrometry (LC-HRAM-Orbitrap-MS) to experimentally identify comprehensive metabolite profiles of BDZ compounds of high prevalence of use and/or clinical and forensic toxicological interest. Licensed BDZs included in the study were alprazolam, bromazepam, clonazepam, delorazepam, diazepam, flurazepam, lormetazepam, lorazepam, midazolam, and triazolam. Designer BDZs were etizolam and bromazolam.

Methods: The study first consisted of an extensive review of the scientific literature about BDZ metabolism and consultation of online databases (e.g. European Medicines Agency, Human Metabolome Database, DrugBank Online, mzCloud) to recognize possible phase I and II metabolites of the selected BDZs. Several pairs of whole blood and urine samples from subjects who had already tested positive for the use of one or more BDZs were then analysed to experimentally verify the metabolic profiles identified in the literature review. Samples (100 mcL) were subjected to protein precipitation with cold acetonitrile:methanol 2:1 (v/v) in the presence of the internal standard

(IS) Nordiazepam-D5. The extracts obtained were subjected to LC-HRAM-Orbitrap-MS analysis using a Thermo Scientific Ultimate 3000 UHPLC system equipped with an Accucore Phenyl Hexyl reversed-phase analytical column coupled to a Thermo Scientific "Q-Exactive Focus" Orbitrap mass spectrometer instrument. MS acquisition was performed in full scan (m/z 70-1000) positive-ion mode at a resolution of 70,000, and subsequent data-dependent acquisition (dd-MS2) confirmation mode (resolution 17,500, isolation window 4.0 m/z, isolation offset 1.0 m/z, normal collision energies 17.5, 35.0, 52.5 eV), according to an inclusion list of about 100 exact mass values, calculated from the elemental composition of MH+ ionic species of about 130 parent BDZs and metabolites, inferred from the relevant scientific literature.

Results & Discussion: Around 130 publications on the metabolism of the selected BDZs, including in vitro and animal studies, have been considered, and at least 6 online databases have been consulted.

As regards sample preparation, protein precipitation was found to be a simple, rapid, and inexpensive procedure that allows exhaustive extraction of both phase I and phase II metabolites, without disrupting the original metabolic patterns.

Eighty-six analytes (12 parent BDZs and 74 phase I and phase II metabolites) were detected and identified following the evaluation of absolute and relative (to IS) chromatographic retention times (RTs), accurate mass measurements and comparison of the experimental and calculated isotopic patterns of the MH+ ionic species in full scan conditions, relative isotopic abundance (RIA, M+2/M+0 ion abundances for Cl- and Br-containing BDZs) error values, accurate mass measurements of MH+ collision-induced product ions following dd-MS2 experiments. Mass accuracy and RIA error values were below 2 ppm and 19% for all analytes, respectively.

From the analytical data, comprehensive and characteristic profiles were defined for each of the 12 BDZs, each with a variable number of analytes ranging from 4 (lorazepam) to 12 (flurazepam), resulting in specific HRAM-MS ion chromatogram layouts. In particular, the discrimination of structural isomers of the hydroxylated phase I metabolites (e.g. 1-hydroxymethyltriazolam and 4-hydroxytriazolam, or 1-hydroxymidazolam and 4-hydroxymidazolam) has been achieved evaluating the characteristic fragmentation patterns. Similarly, phase II metabolites, as O-glucuronides or N-glucuronides, were identified due to characteristic fragmentation patterns matching those of the parent drug or phase I metabolites from which they derived. Also, for BDZs with chiral carbon atoms, pairs of peaks with different RTs but identical MS characteristics were identified, due to the diastereoisomeric R and S forms of the glucuronides. The analytical approach described has also been applied to the analysis of vitreous samples from deceased BDZ users.

Conclusion: The identification of exhaustive and characteristic metabolite profiles through the application of specific ion chromatogram layouts (not only to HR, but also to low-resolution MS acquisition files) increases the power of detection of BDZ intake, making it possible to unequivocally prove, even retrospectively, the consumption of specific BDZs. This is the case, for example, with lormetazepam glucuronides. Since lormetazepam shares several phase I metabolites with lorazepam and delorazepam, the monitoring of lormetazepam glucuronides can be used as evidence of lormetazepam intake. Following the 12 BDZs included in this study, similar profiles can be studied and obtained for other licensed or designer BDZs.

HR P-07

Enhancing the forensic toxicology workflow: Optimization of an UPLC-Q-ToF toxicological screening method of blood samples

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Background & Aims: Untargeted systematic toxicological analysis (STA) is an essential step in routine toxicological casework (post-mortem, drug-facilitated sexual assault, etc.). In our current STA workflow, compounds are identified in biological fluids using ultra-performance liquid chromatography–quadrupole–time-of-flight (UPLC-Q-TOF), and thereafter, confirmation and/or quantification is performed using targeted methods. We aim to evaluate, and if necessary, optimize our UPLC-Q-TOF screening method to minimize the need for additional targeted analysis.

Methods: A 15-minute LC-QTOF (XEVO-G2 XS QTOF) method was based on the Waters UNIFI Forensic Toxicology Application, including a library with over 2000 compounds. Data processing was done via Waters Connect 3.2.2.

Sample preparation (100 µL) consisted of cold acetonitrile (ACN) protein precipitation, followed by evaporation, and reconstitution in 100 µL of 5 mM ammonium formate pH 3.00. The following identification criteria were used: retention time tolerance < 0.5 min; tolerated mass error < 5 ppm; isotope match pattern and intensity < 20%; response intensity > 1,000, fragments found/expected: > 50%. Limits of Identification (LOI) were determined by analyzing standard stock solutions (e.g., benzodiazepines, antidepressants, antipsychotics, amphetamines, cocaine and metabolites, opiates, cannabinoids (121 compounds in total)) in serial dilutions (n=6). Additionally, the effect of adding 20 µL of HCl in isopropanol during evaporation was evaluated both concerning sensitivity, as well

as compound stability. Moreover, matrix effects were evaluated via the assessment of standard solutions spiked with sodium fluoride (SF) blood (concentrations spiked from LOI to 3 x LOI). Experimental analyses were performed in positive polarity except for the analyses of delta-9-tetrahydrocannabinol (THC) and its metabolites.

Results & Discussion: LOIs of 113 compounds from different pharmacological classes were established. The method demonstrated high sensitivity. Using the current identification criteria, 8 compounds were not detected automatically (e.g., benperidol, 7-OH-quetiapine, duloxetine, cloxazolam, desmethylflunitrazepam, loprazolam, THC, and para-methoxyamphetamine). Though THC was never detected, the metabolites THC-OH and THC-COOH were observed. Amphetamine is known to be volatile during evaporation unless acid is added. The addition of HCl during our sample preparation avoided this problem and, in general, improved the LOI of the other tested compounds, except for the benzodiazepines, which were not altered. Moreover, amphetamine identification is complicated due to in-source fragmentation resulting in monitoring via fragments and not the parent ions.

When evaluating the effect of SF matrix, no notable differences were observed regarding the detection of the compounds (no extensive matrix suppression). We aim to further evaluate the potential impact of matrix effects by comparing hemolyzed post-mortem blood to freshly drawn SF blood.

Data interpretation of Q-TOF results requires some time investiment as the importance of each hit in relation to the case needs to be evaluated. The relevance of the compound(s), the detection of metabolites or degradation products versus parent, and the case context and possible various matrices should be taken into consideration. However, having an automatically generated concise report of the Q-TOF screening results helps to get a cost-efficient STA. The automatic identification and subsequent report can give hits for endogenous compounds (e.g., tyrosine, cortisol), and toxicologically less relevant compounds (e.g., nicotine, caffeine). To facilitate interpretation, these compounds were marked on the automatically generated report. The aim of our experiments was to have an idea of the sensitivity of our screening method, to eliminate unnecessary target analyses. It is important to evaluate the reasons why some compounds were not detected using our current processing thresholds and identification criteria. There can be several reasons, e.g., sensitivity, but also acquisition problems (noise, etc.) or processing choices. Therefore, the determination of LOI can also help us to establish an even better quality assurance (QA) program. Based on our evaluation, we aim to expand our current quality control (QC) sample (plasma spiked with bromazepam, trazodone, diazepam, oxazepam, and fluoxetine) and internal standard (ß-OH Theophylline) with additional compounds spanning a larger variety of retention time and masses.

Conclusion: By determining the LOI for frequently encountered toxicologically relevant compounds, we aim to decrease the number of confirmatory analyses needed in our current STA workflow. Adding HCl during evaporation of the extraction solvent showed promising results, and matrix effects seem negligible. An automatically generated concise report remains challenging but can diminish the time spent interpreting results. Getting a better idea of the method's sensitivity and parameters impacting the detection ability of compounds makes it possible to establish adequate QA measures. This finally leads to more confidence in the behavior of the system during a run and thus in the final results.

Toxicometabolomic analysis of the synthetic cathinone N-ethyl pentedrone using zebrafish model

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Background & Aims: New Psychoactive Substances (NPS) are compounds not controlled by international conventions and which have central nervous system activity. Among the NPS classes, synthetic cathinones (SC) stand out as one of the most numerous groups. Although the SC N-ethyl pentedrone (NEP) has been associated with several intoxication cases, research on its toxicity remains unclear. Understanding the metabolism of xenobiotics can be a crucial way for predicting their effects, toxicities, and potential treatments. Alternative models such as zebrafish are gaining popularity due to their genetic and physiological similarities to mammals, as well as their cost-effectiveness and rapid development. The Zebrafish Water Tank (ZWT) model has emerged as a valuable tool for studying drug metabolism, providing reliable insights about metabolites identification. Additionally, toxicodynamic-based metabolomics studies allows qualitative and quantitative analysis of small molecules related to cellular toxic events. Such an approach aims to identify exposure biomarkers, propose mechanisms of action, and develop prospective antidotes. The aim of this study was to investigate the metabolic profile and toxic effects of NEP in zebrafish brain tissues.

Methods: Zebrafish exposures to NEP were adapted from ZWT model, described by De Souza Anselmo et al. (2018). Six-months old male zebrafish (N = 40) were transferred to five tanks containing 200 mL of reconstituted water (RW) (eight animals per tank). These tanks were divided into two groups: two negative controls, constituted by sixteen animals without drug exposure; and three exposure tanks ([NEP] = 0.5 µg/mL), constituted by twenty-four animals exposed to NEP. After an 8-hours exposure, animals were euthanized, their bodies were decontaminated with RW, and their brains were removed. Pooled samples were formed (five brains per sample), yielding four NEP-exposed and three non-exposed zebrafish pooled brains. Metabolites were extracted by a homogenization protocol using methanol added to internal standard (methamphetamine-d5, 10 µg/mL). Blank samples were also prepared following the same procedure, but without tissues. Aliquots of all samples were pooled for metabolomics quality control (QC) preparation. Samples were vortexed, centrifuged, and the supernatant evaporated. Finally, samples were resuspended in 80 µL in water:acetonitrile (98:2, v/v) and injected 5 µL into a liquid chromatography-tandem high resolution mass spectrometry system. All experiments were approved by the Ethical Committee for Animal Research from UNICAMP (protocol 6253-1/2023). For NEP metabolites identification, identified ions observed in MS mode corresponding to theoretical nominal mass compatible with phase I and phase II metabolism reactions were considered and investigated. Identification criteria were: (I) precursor ions mass error < 5 ppm, (II) fragments mass error < 15 ppm, and (III) mass spectrum with at least two structurally consistent ions. For toxicometabolomics analysis, data were processed using MS-Dial for peak picking, chromatogram deconvolution, alignment, and integration, using public databases for metabolomics data. MS-FINDER was also employed for improving metabolites annotation. The obtained matrixes were manually reviewed in order to remove blank background and filtered by QC features with coefficient of variation > 20%. Processed data were analyzed through Metaboanalyst 5.0 for uni- and multivariate analysis.

Results & Discussion: Seven NEP metabolites were found in zebrafish brain tissues. Metabolite 1 (M1) was characterized by a N-dealkylation and was the most abundant metabolite while M2 was produced by a beta-ketone reduction. Similarly, M1 also undergone to a beta-ketone reduction, producing M4 – the second most abundant NEP metabolite. Hydroxylation reactions were also observed, occurring in the aromatic ring (M3) and in its alkylic side chain (M5). Moreover, two phase II metabolites were observed, in which M6 was produced by an aromatic hydroxylation followed by O-glucuronidation, and M7 by M6 N-dealkylation. None of the metabolites found in the exposure tanks were observed in the negative controls. The performance of toxicometabolomics analysis were achieved by evaluating QC samples through PCA. The analysis reached considerable reliability throughout all injections, ensuring a suitable comparison along the samples. Differences in the metabolome were highlighted between exposed and non-exposed animals using OPLSDA models, in which discriminant metabolites were observed, potentially associated to NEP toxic effects. Univariate analysis also pointed out to statistically significant and differentially expressed metabolites (p-value < 0.05 and FC > 2). Eighteen metabolites were found upregulated and seventeen downregulated in exposed group (ESI+). Moreover, twenty-two metabolites were found upregulated and five downregulated in exposed group (ESI-). The discriminant metabolites corresponded to pathways related to metabolism and release of neurotransmitters, such as glutamate, homovanillic acid and kynurenine; lipid metabolism, such as O-phosphocholine, lysoPC (18:1); and energetic metabolism, such as citrulline. These molecular events potentially reveal important features regarding NEP neurotoxicity.

Conclusion: In conclusion, our study elucidates NEP neurotoxicity in zebrafish, identifying seven NEP metabolites and associated metabolic pathways. Notably, M1 predominated, while M6 and M7 reflected phase II metabolism. Multivariate analyses highlighted significant alterations between exposed and non-exposed groups, implicating neurotransmitter release, lipid and energy metabolism, and oxidative stress. These findings enhance our understanding of NEP toxicity mechanisms, being useful for toxicological assessments.

HR P-09

Two cases of illicit drug adulterant-related intoxications in Italy: Trends of a beyond suspicion public health threat

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Background & Aims: Drug of abuse adulteration is the practice of adding active principles to the primary psychoactive drug during the manufacturing process to increase drug dealers' profit by obtaining a more potent mixture or different recreational effects at a lower price. However, unexpected toxicity may result due to drug-drug interactions or pharmacological enhancement. A wide panel of pharmaceutical drugs, veterinary drugs and New Psychoactive Substances (NPS) have been recently detected as drug adulterants on the illicit drug market, exposing the consumers to unsuspected harm. Recently, the veterinary drug xylazine, a potent central α-2 adrenergic agonist, raised the concerns of international agencies and governments due to its involvement in an increasing number of fentanyl-related deaths as the principal drug adulterant. In the summer 2023, the Italian Early Warning System ("Sistema Nazionale di Allerta Precoce", SNAP) on NPS investigated a series of drug-related fatalities (n=3)

that occurred in Sardinia. Among those, a 33-year-old Italian male with a documented history of drug abuse was found dead in a public area in the late morning during a summer weekend (case #1). The preliminary screening of postmortem blood and urine samples revealed the presence of opioids. Further toxicological analysis disclosed the detection of xylazine and morphine in this fatality, suggesting the exposure to "tranq-dope": xylazine-adulterated heroin. After the III grade Alert issue, another suspected case of xylazine non-fatal intoxication (case #2) from Naples was submitted to the SNAP due to the occurrence of unexpected ulcerations on the legs of a drug consumer. In this case, a habitual drug user under opioid substitution therapy, presented extensive ulcerations of the legs, similar to those reported following unintended exposure to xylazine-adulterated opioids. Urine and hair were collected for toxicological screening.

Methods: After spiking with 5 µL of internal standard (6-monoacetyl-morphine-d3, benzoylecgonine-d3, methadone-d3, and ketamine-d4 at 1 µg/mL in acetonitrile), 100 µL whole blood was protein precipitated with 250 µL acetonitrile containing 0.2% hydrochloric acid. The sample was then centrifuged and the supernatant was evaporated to dryness under nitrogen. After reconstituting with 100 µL of mobile phases A:B 90:10 (v/v), 5 µL was injected into the chromatographic system. A volume of 50 µL urine was spiked with 5 µL of the internal standard mixture and incubated with 10 µL β-glucuronidase for 2 h at 50°C. Then, the samples were mixed with 125 µL acetonitrile containing 0.2% HCl and centrifuged, and the supernatants were evaporated to dryness under nitrogen. After reconstituting with 100 µL of mobile phases A:B 90:10 (v/v), a volume of 5 µL was injected into the chromatographic system. An aliquot of 20 mg of hair was extracted 2 mM ammonium formate in MeOH:ACN:H2O (25:25:50 v/v/v) + 0.1% formic acid, dried under nitrogen, reconstituted in mobile phases and injected in the chromatographic system. Screening analysis was performed by a validated LC-HRMS/MS method with a DIONEX UltiMate 3000 liquid chromatograph coupled with a Thermo Scientific Q Exactive mass spectrometer equipped with a heated electrospray ionization source. The same sample preparation protocol and chromatographic conditions were applied for the xylazine quantification in LC-HRMS/MS. Confirmatory quantitative analyses of classic drugs of abuse were performed in gas chromatography-mass spectrometry.

Results & Discussion: Concerning case #1, the toxicological screening revealed the presence of opioids, methadone, benzoylecgonine and benzodiazepines in whole blood, while opioids, cocaine and benzoylecgonine, xylazine and 4-OH-xylazine, benzodiazepines and 2-ethylidene-1,5-dimethyl-3,3-diphenyl pyrrolidine were detected in urine. Xylazine concentrations were 72.6 ng/ml in blood and 104.6 ng/ml in urine, while morphine concentrations were 235.7 ng/ml in blood and 59.1 ng/ml in urine. Although morphine blood concentration might have been lethal, the cause of death was attributed to polydrug intoxication from the combination of central nervous system depressants, i.e., opioids and xylazine. In case #2, urine analysis surprisingly revealed the consumption of cocaine, methorphan and tramadol besides the declared heroin and buprenorphine use. The chronic consumption of cocaine was confirmed by hair analysis. However, xylazine and its metabolite were detected in none of the samples. Instead, further analyses revealed the presence of levamisole in all the samples. Indeed, the chronic exposure to levamisole determined the peripheral vasculitis resulting in extensive ulcerations.

Conclusion: For the first time, the detection of xylazine in a heroin-related fatality was reported in the European Union through the Early Warning System. Adulterants complicate the toxicology laboratory efforts against the drug and NPS issues, posing ever-new unsuspected challenges. Since 2009, the SNAP aims to monitor the NPS offer on the regional drug market and the new drug consumption trends to inform the collaborative centrrs on new threats. In 2023, the SNAP issued 25 alerts, 15 informative documents and 21 reporting forms from the European Early Warning System. Although NPS represent its main focus, the SNAP played a crucial role in raising attention to drug adulteration practices as a public health threat.

Urinary forensic toxicology data independent analysis screening: Using high resolving power multi-reflecting time-of-flight mass spectrometry

HR P-10

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Background & Aims: Laboratories are frequently required to perform broad screening techniques on complex biological samples to identify drugs of abuse, prescribed agents and other toxicants. Broadband data-independent analysis (DIA) has been previously applied for non-targeted screening of forensic samples. The analytical strategy used high resolution mass spectrometry (HRMS@ 20,000 FWHM) and enabled collection of an unbiased dataset, providing a complete profile of the sample complement, including precursor and fragment ions.

Here, we demonstrate a further enhancement of DIA specificity, through use of a high mass resolving power (>200,000 FWHM) hybrid quadrupole multi-reflecting time-of-flight mass spectrometer (Q-MRT MS). Analysis of anonymised urine samples shows enhanced analyte ion selectivity in complex matrices, improved mass accuracy and analyte identification confidence.

Methods: Broadband DIA using alternate scanning precursor/fragment ion data acquisition was performed using Q-MRT MS (system resolving power >200,000 FWHM) acquired using positive electrospray mode ionization. An acquisition rate of 10 Hz and a collision energy ramp of 10-40 eV were applied. Human urine samples were analysed, using reversed phase chromatography, comprising gradient elution of a 2.1 mm x 150 mm, 1.8 µm C18 column. The column was maintained at 50°C and eluted with a mixture of (A) 5 mM ammonium formate in water, pH 3.0 and (B) acetonitrile with 0.1% formic acid delivered at 0.4 mL/min. The run time was 15 minutes and an injection volume of 5 µL was utilised. Authentic urine samples were diluted 1:10 (into water) prior to the analysis.

Results & Discussion: Non-targeted data acquisition has been performed for a forensic system suitability test mix (SST) and a series of authentic human urine samples. Post-acquisition processing involved comparison with the comprehensive Waters Forensic Toxicology library of 1975 toxicologically relevant compounds, including illicit drugs, prescription and over-the-counter medications, and pesticides.

For the 10-component SST mix (250 pg/µL) mass error (RMS) of 522 part-per-billion (ppb) was obtained, where data processing tolerances of tr (±0.35 min) and mass accuracy (±2ppm), diagnostic fragment ion count of at least 1 and expected fragment ion mass tolerance of 0.2 mDa were applied. All SST analytes were identified using the more stringent data processing parameters. As an example, for clozapine, precursor m/z 327 [397 ppb (184,000 FWHM)] and fragment ions m/z 270 [411 ppb (190,000 FWHM)], m/z 227 [236 ppb (183,000 FWHM)], m/z 192 [382 ppb (178,000 FWHM)] have been observed.

The same parameters were also applied when processing the urine samples and comparing against the library. The sub-ppm mass measurement and high specificity attained for the SST constituents afforded increased confidence for identifications made in the authentic samples, which included illicit and prescribed drugs, their metabolites, as well as dietary/endogenous constituents. For example, in authentic sample "103", methadone [m/z 310 (290 ppb)] EDDP [m/z 278 (350 ppb)], methamphetamine (m/z 150 (-520 ppb)), morphine (m/z 286 (930 ppb)), normorphine [272 (m/z (920 ppb)], codeine [m/z 300 (-290 ppb)], norcodeine [m/z 286 (-570 ppb)], nicotine [m/z 163 (-460 ppb)], cotinine [m/z 177 (-1090 ppb)], caffeine [m/z 195 (110 ppb)], and theophylline [m/z 181 (960 ppb)], have been identified.

Conclusion: The high resolving power of the Q-MRT MS enhances ion selectivity and detection of analytes in complex biological matrices. Mass accuracy specificity, typically achieving ppb, can be utilised to in conjunction with stringent data processing tolerances to increase confidence in analyte identification, improve analysis efficiency and enhance analytical research involving small molecules in an ever evolving drug landscape. Illicit drug substances were detected in all samples tested using retention time, the presence of a precursor ion and a fragment ion and ppb mass accuracy.

HR P-11

Unraveling the cannabis plant metabolome: A novel analytical framework for the comprehensive chemical profiling of cannabis utilizing GC-HRMS analysis

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Background & Aims: Cannabis sativa L., commonly known as the cannabis plant, has historically been utilized for both recreational and medicinal purposes. Its significance has increased in recent years, especially focusing on its primary chemical constituents, Δ9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD). Although traditional analytical studies focus primarily on these cannabinoids, the cannabis plant comprises over 600 identified compounds, including around 150 cannabinoids, some 200 terpenes, numerous flavonoids, and other lesser-known substances. This complex composition requires a comprehensive chemical profiling of cannabis, that goes beyond just THC and CBD content. This need has become particularly apparent following recent legislative changes worldwide, such as the amendment in Swiss law that facilitates pilot programs for distribution of non-medical cannabis and easier access to medicinal cannabis. These developments have led to the presence in the market of both legally sold THC-rich cannabis under pilot programs and illicitly sold products, increasing the importance of distinguishing between various cannabis offerings. Our project aims to address this need by developing an analytical technique that can characterize cannabis samples based on their entire metabolome. This approach not only considers the primary phytocannabinoids but also minor and previously unidentified components, thereby assigning each cannabis sample a unique fingerprint for differentiation and classification beyond traditional chemical phenotypes.

Methods: Our methodology involved the selection of 35 CBD-type cannabis strains grown under three different conditions (greenhouse, outdoor or indoor), from which three flower clusters were blended into a uniform mixture. From this mixture, 100 mg samples were extracted using ethanol and then diluted by a factor of 50, with each sample preparation conducted in triplicate. Additionally, control samples without cannabis (negative controls), a combined sample from all varieties (pool sample), and dilutions of this pool were also prepared. To conduct an untargeted analysis, 1 µl

of each sample was injected into a TRACE 1300 GC system connected to a Q Exactive GC Orbitrap mass spectrometer. This system, using a TraceGOLD TG-5SilMS capillary column, operated in electron ionization positive mode, capturing a full scan of ions over a mass range of m/z 40-500. Data pre-processing started with MS-DIAL software. A LOESS batch correction was performed using nPYc and the data was filtered using the following criteria: the relative standard deviation (RSD) of a feature in the pool samples should not exceed 25%; the Pearson correlation in the pool dilution samples should be higher than 0.8; and the RSD of a feature within the real samples should be at least twice as high as in the pool samples. Statistical analysis was performed using the MetaboAnalyst 6.0 platform, employing normalization using the pooled samples, square root transformation, and automatic scaling to facilitate comparability across samples. Compound identification was performed using reference standards and the NIST library.

Results & Discussion: Data pre-processing yielded 109 features defined by molecular mass (m/z) and corresponding retention time. Principal Component Analysis (PCA) revealed distinct groupings of the varieties within the dataset, as well as groupings of the growing conditions. A K-Means clustering analysis grouped these samples into four distinct clusters, indicating substantial variability in chemical composition of the samples. Excluding the features representing THC and CBD still allowed distinct grouping of the varieties as well as the growing conditions. Important features were identified as compounds corresponding to the terpene group, such as e.g. humulene, α-terpineol, β-caryophyllene and caryophyllene oxide. These findings highlight the complexity of cannabis chemistry and the potential of GC-MS analysis combined with advanced statistical methods to unravel it. The identified key compounds not only shed light on the chemical diversity within cannabis samples but also pave the way for further investigations into their specific functions and effects. This analytical and statistical approach demonstrates its efficacy in forensic and pharmaceutical cannabis research, offering insights that can guide the cultivation, processing, and application of cannabis-based products.

Conclusion: In conclusion, this study introduces a novel analytical framework for the comprehensive chemical profiling of cannabis employing GC-HRMS analysis and advanced statistical techniques. Our results demonstrate the importance of plant metabolites other than THC and CBD regarding the chemical phenotype of cannabis. In addition, they provide a deeper understanding of the chemical complexity of cannabis and contribute to the differentiation and classification of cannabis samples for medical, legal, and forensic purposes. As the legal landscape surrounding cannabis continues to evolve, the demand for precise, comprehensive analytical techniques grows. Our study's approach to characterizing the entire metabolome of cannabis samples represents a significant advancement in meeting this demand.

Poster gallery – DIV F-P-1 to P-32

16:00 - 16:30 Thursday, 5th September, 2024

Adverse analytical finding for ostarine in a doping context explained by contamination by body fluids

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Background & Aims: Ostarine, also known as MK-2866 or enobosarm, is a selective androgen receptor modulator (SARM). It has anabolic properties and as such is widely used in doping, accounting in 2021 for 25% of the adverse analytical findings (AAF) among the class S1.2 "Other anabolic agents" of products banned by the World Anti-Doping Agency, to which it belongs. But in some cases, it can be responsible for an AAF following contamination. We report the case of an athlete who contaminated herself by exchanging body fluids while kissing her boyfriend, who took 1 mL per day of a 25 mg/mL solution of MK-2866 for 9 days prior (D0) to the athlete's AAF (D9, urinary concentration evaluated at 13 ng/mL) without her knowledge.

Methods: Both subjects came to our lab 4.5 weeks after the AAF (D35). As ostarine was still administered at that time, only urine was collected, and urine, fingernails, toenails and hair were collected after 6 days of discontinuation (D41). Oral fluid was not collected. The athlete's hair was black and slightly frizzy. Six segments of 2 cm then 7 x 3 cm (33 cm) were analysed. The boyfriend's hair, light-brown, were analyzed on 4 x 2cm.

A controlled transfer study was carried out at their house 12 days (D47) after discontinuation (urine concentrations returned to negative level). After administration of 17 mg (the 25 mg/mL vial having been controlled at 17 mg/mL), urine samples were taken from the boyfriend and the athlete (n=10 for each, from 1h to 25h and 20min after administration) after they had been living normally with each other (regular kissing in particular). Pillowcases of each subject, and the athlete's hairbrush were collected with a swab.

Ostarine was analyzed with a validated LC-MS/MS method.

Results & Discussion: Hair concentrations in the athlete increased from 2 pg/mg on the first segment to 17.8 pg/mg on the last segment and those of the boyfriend from 65 to 143 pg/mg. These gradients of concentration in the hair's athlete and in her boyfriend were compatible with external contamination of the hair, confirmed by analysis of hair washing baths, pillowcases (150 pg on each), and the hairbrush (250 pg). Fingernails were also contaminated, with 21 pg/mg in the athlete and 1041 pg/mg in the boyfriend, with highly contaminated washing baths, while toenails were less contaminated, with 2 pg/mg in the athlete and 17.3 pg/mg in the boyfriend. In the first urine collected (D35), a value of 3690 ng/mL in the boyfriend and 5.7 ng/mL in the athlete were measured. After 6 days off, these concentrations were 3.3 ng/mL and 0.1 ng/mL, respectively.

After controlled administration, the boyfriend's urine concentrations ranged from 681 ng/mL to 12822 ng/mL (Tmax=8:30 hrs), and the athlete's from 0.3 ng/mL to 13 ng/mL with Tmax=8:30 hrs, i.e. at 22:30 hrs, which corresponded exactly to the time of collection of the urine that showed AAF, with a similar concentration. According to the area under the curve obtained in each subject, the dose ingested by the athlete was estimated at 15 µg, which is an ineffective dose.

Conclusion: These results demonstrate the transfer of ostarine via body fluids between two subjects, with a high risk of AAF in an athlete, as observed in our case.

DIV F-P-02

Evaluating occupational lead exposure in an Algerian lead battery recycling plant: Significance for worker health and safety

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Background & Aims: Lead is undoubtedly one of the oldest occupational toxins. Approximately 50% of the global lead production comes from battery recycling. While the collection rate of automotive batteries reaches 97% in the United States and 85% in France, it is entirely unknown for Algeria due to the absence of official statistics. Algeria generates approximately 2.1 million tons of used automotive batteries annually, of which only 30% are recovered and recycled. Lead poisoning is a concerning occupational disease and a pervasive environmental threat to the health of exposed individuals. While lead exposure and release rates are carefully controlled in developed countries, they can be considerably higher in low or middle-income countries. It is in these latter countries where the highest burden of morbidity is observed. Lead poisoning is considered the best-characterized occupational disease; this poisoning, especially in the battery sector, is extremely common worldwide, and Algeria is no exception. However, little information is available on the level of lead exposure among workers in this sector in Algeria and on the effects of this metal on the health of Algerian workers. Through this study, we report on an exploration of occupational lead exposure among workers in a lead battery and accumulator recycling unit in Algeria.

Methods: This descriptive study aimed to evaluate the occupational lead exposure of workers in a lead battery recycling plant located in the SETIF province of eastern Algeria by measuring lead concentrations in their blood. Participants, all of whom provided informed consent, were selected from the plant's workforce during routine medical check-ups. Blood samples were collected following established protocols and stored at -20°C before being transported to the Department of Analytical Sciences at the Hubert Curien Multidisciplinary Institute (IPHC) in Strasbourg, France. Metal analysis to determine lead concentrations was conducted using Triple Quadrupole Inductively Coupled Plasma-Mass Spectrometry (ICP-QQQ MS/MS) Agilent 8900. The results were subsequently evaluated to assess the workers' levels of lead exposure, providing valuable insights into occupational health and safety within this industry.

Results & Discussion: Our investigation uncovered elevated levels of lead in the bloodstream of workers at this facility. The highest mean and median concentrations were detected in the "smelting" workshop, where molten lead is refined (753.83 µg/L and 714.65 µg/L, respectively), followed by the furnace, grinder, maintenance, versatile, acid neutralization station, stock, and administration, in descending order. In the battery recycling workshops, the average blood lead level was 651 ± 221.4 µg/L (with a maximum of 1027 µg/L), while among office employees, particularly those in administration, it was notably lower at 320.6 ± 184.4 µg/L. Excluding the administration staff, there was no significant disparity in blood lead levels observed among the recycling workshops; the rank comparison test

yielded a non-significant result (p=0.34). However, a notable difference was noted between the administration staff and other personnel. This discrepancy can be attributed to the operational layout of the recycling facility, where all tasks are conducted within a single open space devoid of physical partitioning between workshops and lacking an effective air treatment system.

Conclusion: In conclusion, our study highlights the substantial occupational lead exposure among workers at the lead battery recycling plant. The highest lead concentrations were observed in the "smelting" workshop, indicating a heightened risk of lead toxicity among workers involved in the refinement of molten lead. While significant differences were noted in blood lead levels between administration staff and other personnel, suggesting variations in exposure levels across different roles within the facility, it's imperative to recognize the overall elevated risk of lead poisoning among all workers. These findings underscore the urgent need for comprehensive occupational health and safety measures, including the implementation of effective ventilation systems and the provision of appropriate personal protective equipment, to mitigate the adverse health effects associated with lead exposure in such industrial settings. Additionally, regular monitoring of blood lead levels and adherence to stringent safety protocols are essential to safeguard the health and well-being of workers in the lead battery recycling industry.

Be aware of (R)-methamphetamine: negative immunoassay vs. positive confirmation analysis

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Background & Aims: Methamphetamine is a psychoactive amphetamine-type stimulant that exists in two enantiomeric forms: (S)-methamphetamine and (R)-methamphetamine. However, the (S)-enantiomer is more potent than the (R)-enantiomer. Racemic mixtures of both enantiomers or enantiopure (S)-methamphetamine are common forms of illicit methamphetamine encountered in Europe. This study reports two driving under the influence (DUID) cases of proven enantiopure (R)-methamphetamine use, both of which remained undetected by immunoassay screening.

Methods: Immunoassay screening for amphetamines in plasma was performed on a Beckman Coulter AU 480 auto analyzer by using Cloned enzyme donor immunoassay (CEDIA®) Amphetamine OFT and CEDIA® Amphetamine/Ecstasy reagents. Cut-off levels were adjusted for forensic purposes to detect amphetamine and methamphetamine plasma concentrations of at least 25 ng/mL with a sensitivity of not less than 90%. Plasma samples were classified as "positive" if their measurement lead to a concentration exceeding the validated in-house laboratory cut-off concentration (9.0 ng/mL, CEDIA® Amphetamine OFT; 21 ng/mL, CEDIA® Amphetamine/Ecstasy). To determine immunoassay cross-reactivity, each enantiomer of amphetamine and methamphetamine was spiked at different concentrations (ranging from 1.0 ng/mL to 1,000 ng/mL) in drug-free plasma. Plasma samples of DUID cases were additionally analyzed using a validated liquid chromatography-tandem-mass spectrometry (LC-MS/MS) method. Linearity of the method was demonstrated in the range of 5.0 ng/mL to 300 ng/mL ($R2 > 0.99$). The limit of detection (LoD) was determined as follows: (S)-amphetamine 1.0 ng/mL, (R)-amphetamine 2.2 ng/mL, (S)- and (R)-methamphetamine 0.6 ng/mL. Matrix effects demonstrated low ion suppression, but were negligible (85-90%). Both methods (immunoassay and LC-MS/MS) were validated according to the guidelines of the Society of Toxicological and Forensic Chemistry (GTFCh).

Results & Discussion: No positive amphetamine results (CEDIA® Amphetamine OFT) were obtained for spiked samples containing either (S)- or (R)-methamphetamine at any of the tested concentrations. Plasma samples spiked with (S)-methamphetamine and (S)-amphetamine tested positive for methamphetamine (CEDIA® Amphetamine/Ecstasy) at 15 ng/mL and 25 ng/mL, respectively. For (R)-amphetamine and (R)-methamphetamine, positive results were observed at 200 ng/mL by the CEDIA® Amphetamine/Ecstasy assay. In both DUID cases, the use of methamphetamine or amphetamine was reported to have occurred close to the time of the incident. Immunoassay screening, however, was negative in both cases. Quantitative analysis revealed (R)-methamphetamine concentration of approx. 320 ng/mL (case 1) and 170 ng/mL (case 2), with low (R)-amphetamine concentrations as well. In both plasma samples, despite potentially significant levels of methamphetamine, immunoassays did not indicate methamphetamine presence due to the greater sensitivity for the (S)- compared to the (R)-enantiomer.

Conclusion: Users of immunoassay drug tests should be aware that inconspicuous immunoassay findings may be due to the considerably enantiomer-dependent sensitivity and concentration-dependent cross-reactivity of the applied drug of abuse assay for amphetamine or amphetamine-type stimulants.

DIV P-04

Medical fitness-to-drive in long-term users of zolpidem

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Background & Aims: Driving is a complex task that places demands on many cognitive skills which can be impaired by psychoactive substances. Benzodiazepines and non-benzodiazepines Z-drugs are the most commonly used medication in the treatment of chronic insomnia and bind to GABAA receptors in the brain promoting anxiolytic and sedative effects but also psychomotor and cognitive impairment.

Epidemiological data has shown that acute benzodiazepine use is associated with psychomotor and cognitive impairment as well as an increased risk of being involved in traffic accidents and performing unsafe driving behavior.

However, it has also been suggested that partial tolerance to these adverse effects occurs after long-term use (three years).

Furthermore, former reports also indicated that long-term benzodiazepines use might increase the risk of permanent cognitive impairment, and that long-term use should be avoided in the elderly.

The aim of this study was to evaluate whether cognitive impairment influencing fitness-to-drive occurs in long-term zolpidem users (more than two years of 10 mg stable usage) using neurocognitive paper-and pencil tests.

Methods: The individuals recruited for this study included 10 middle-aged male individuals suffering from chronic insomnia, who were long-term users of 10 mg zolpidem (more than two years of stable usage), with a frequency of administration of at least five times a week, with no history of secondary insomnia.

The individuals recruited for this study underwent fitness-to-drive medical assessment requested by the authorities due to a traffic offence in the absence of traffic accidents, where they also underwent neurocognitive paper-and pencil tests.

Results & Discussion: Overall, cognitive performance was not significantly affected for any of the administered paper-and pencil tests in any of the recruited participants when compared to reference values (scores provided in the reference populations).

The obtained data seem to indicate that impairing effects of long-term zolpidem use on cognitive performances (paper-and pencil tests) may mitigate over time following long-term use of two years. Furthermore, there was no evidence of permanent cognitive impairment after prolonged use and no evidence of residual daytime sedation 12 hours after the last 10 mg dose administration.

Conclusion: Regarding traffic safety, these results seem to comfort the ICADTS classification of zolpidem as category I when driving occurs at least 10 hours post-dose.

However, several limitations in our study deserve to be mentioned.

First, the individuals recruited represent a relatively homogenous sample due to strict inclusion criteria. Secondly, the limited sample size and the absence of comparative data at treatment initiation do not allow the results to be generalized and inferred with certainty.

DIV P-05

Simultaneous analysis of ultrashort-chain, short-chain, long-chain, and alternative PFAS in human plasma/serum

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Background & Aims: The per- and polyfluoroalkyl substances (PFAS) targeted for analysis in blood may vary based on the specific concern in a given region or population. Some commonly analyzed PFAS range from short- to long-chain compounds (C4 – C10). The ultrashort-chain (USC) PFAS with carbon chain lengths of shorter than C4 have become a major concern due to their prevalence and high levels of occurrence in environmental aquatic systems. Several studies have indicated a rapid increase in environmental concentration of USC PFAS, raising the concern of elevated human exposure. The measurement of USC PFAS in blood can not only monitor the human exposure but also provide a tool for studying the potential risks associated with USC PFAS exposure.

Methods: As it is almost impossible to get PFAS-free human serum, accuracy and precision testing was conducted using fetal bovine serum (as it was identify as lacking all analytes except TFA) fortified with C1 to C10 carboxylic acid and sulfonic acid PFAS. A 100 µL aliquot of serum sample was mixed with both extracted and non-extracted internal standards, along with 200 µL of methanol containing 1.5% formic acid. After centrifugation, 5 µL of supernatant was injected onto a polar-embedded reversed-phase column (Restek Ultra IBD, 100 x 2.1 mm) for

chromatographic separation followed by MS/MS detection (Waters I-Class/Xevo TQ-S). Calibration standards were prepared in the range of 0.05 to 40 ppb in reverse osmosis water containing phosphate-buffered saline. The established method was then applied to measure PFAS in various human serum and plasma samples, including a NIST 1950 reference human plasma. For method accuracy and precision testing, a charcoal-stripped fetal bovine serum (FBS) was chosen due to its absence of C1 to C10 PFAS, except for TFA. Therefore, a TFA isotope, 13C1-TFA, was implemented as a surrogate to test the method accuracy for TFA in the serum sample. Additionally, ten isotopes of C3 to C10 PFAS were added to the samples at 1 ppb to serve as extracted internal standards to verify the accuracy of the entire workflow. Calibration standards were prepared in reverse osmosis water due to its cleanliness for all analytes. Phosphate-buffered saline was incorporated into the calibration standard solution to obtain the similar chromatographic performance between standard and sample solutions. Three fortification levels from 0.4 to 30 ppb were tested for method accuracy and precision.

Results & Discussion: The high polarity of ultrashort-chain PFAS poses a challenge to current analytical practices based on reversed-phase liquid chromatography due to insufficient chromatographic retention. This study demonstrated that a polar-embedded reversed-phase column allows for the simultaneous analysis ultrashort-chain and long-chain PFAS. Satisfactory retention was achieved for C1 to C3 compounds, including trifluoroacetic acid (TFA), perfluoropropanoic acid, trifluoromethanesulfonic acid, perfluoroethanesulfonic acid, and perfluoropropanesulfonic acid. The analytes in this study included C1 to C10 carboxylic acid and sulfonic acid PFAS, along with four alternative compounds: hexafluoropropylene oxide dimer acid (HFPO-DA), 4,8-dioxa-3H-perfluoro-nonanoic acid, 9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid, and 11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid. With quadratic regression (1/x weighted), all analytes exhibited satisfactory linearities with r2 >0.995 and deviations <20%. The calibration ranges differed for various analytes, spanning from 0.05 – 40ppb, 0.1 – 40 ppb, or 0.25 – 40ppb. Due to weaker ionization, TFA and HFPO-DA exhibited a linear range of 0.5 - 40 ppb. All analytes and extracted internal standards had recovery values of within 20% of the nominal concentration across all fortification levels. Satisfactory method precision was demonstrated with %RSD values <12%.

The established workflow was employed for the analysis of two NIST standard reference materials (SRMs), namely NIST 1950 for human plasma and NIST 1957 for human serum. These SRMs are characterized by known concentrations of six or seven short-chain and long-chain PFAS. Six preparations of each SRM were subjected to the analysis with the developed LC method. The results indicated that all measured EIS concentrations fell within 20% of the nominal concentration. Moreover, results illustrate that the averaged experimental concentrations of most PFAS closely matched the reference concentrations, with deviations within 20%. Although the measured PFNA concentration in NIST 1957 exhibited a slightly higher deviation of 26%, it remained within the deviation range of the reference concentration. These results demonstrated that the established method was suitable for accurate measurement of PFAS in both human plasma and serum.

In addition to the reference PFAS, other measurable PFAS were also reported. Regarding to USC PFAS, TFA was commonly found in plasma and serum, whereas PFPrA and TFMS were quantifiable in plasma and serum, respectively. As discussed earlier, TFA contamination is prevalent in various lab materials. Given the challenge of controlling the material used for the collection of blood samples, it's possible that the measured TFA concentration may not solely originate from the blood itself.

Conclusion: A unique method was developed to enable simultaneous biomonitoring of USC PFAS and commonly analyzed C4 to C10 compounds.

First identification of leonurine in preparations of flowers and leaves from Leonotis leonurus, a cannabis-like psychoactive plant.

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Background & Aims: In recent years, the number of websites and forums focused on drug-use experiences has increased. Besides the new psychoactive substances (NPS), new compounds have been tested by several communities. Many of these were derivatives of common plants which psychotropic use is not fully studied. Anyway, a lot of users describe their own experience of consumption in an almost scientific manner. Leonotis leonurus (L.) R. Br. is one of the most popular mentioned plants. It is a subtropical shrub belonging to the Lamiaceae family also known as "wild dagga" or "wild Cannabis". L. leonurus is widely distributed in South Africa where it is frequently used in ethnopharmacological practices to treat asthma, viral hepatitis, and as diuretics. Dried leaves and flowers are smoked to relieve epilepsy and as a treatment for partial paralysis. In addition to the traditional uses mentioned above, L. leonurus is marketed for its alleged Cannabis-like psychoactive effects.

However, little is known about the active substances in the leaves and flowers of L. leonurus.

Leonurine, a guanidinic alkaloid of L. japonicus is supposed to induce many psychoactive effects, even if its presence in L. leonurus has not been verified yet. This study aimed to identify, for the first time, leonurine in preparations (infusion and smoke) of flowers and leaves from L. leonurus.

Methods: Five packs (5 g each) of dried flowers were bought on a website. Leaves (21 g) were collected from 4 plants of L. leonurus acquired from a nursery and then air-dried in our laboratory. An LC-MS/MS analysis was developed for the detection and quantification of leonurine. Chromatographic run was performed with a Zorbax C18 column (2.1×50 mm, 1.8 µm). Acquisition was in positive ionization mode for the multiple reaction monitoring (MRM) transitions 312→181, 114 m/z. Infusion was prepared by adding boiling water (10 mL) to 200 mg of vegetal materials (dried flowers and leaves). The thermostability of leonurine was also tested by adding the substance to infusions prepared at different temperatures. For smoking, 200 mg of vegetal materials were put on a smoking pipe. The aspiration section was connected to a needle inserted in a vacuum vial containing dimethyl sulfoxide (DMSO, 5 mL). The breathing act was simulated by a vacuum system. A 1-ml aliquot of DMSO was dried and then injected into the LC-MS/MS system.

Results & Discussion: Regarding infusions, leonurine extracted amounts were 32.68 ng (flowers) and 55.02 ng (leaves). Temperature proved to be a key factor as we observed a reduction of leonurine presence up to 90%. Leonurine was not found in the leaves' smoke, while in the flowers' one was measured at 2.64 µg. This difference may be due to the lower combustion rate of flowers which leads to a slower and more effective release of the vapors, and substances as well.

Conclusion: For the first time, in this study, we demonstrated the presence of leonurine, a well-known psychoactive substance, in the flowers and leaves of L. leonurus. Moreover, we observed that infusion allowed to extract leonurine from both vegetal materials; by smoking, leonurine was found only in the flowers' smoke and in higher amounts. More preparations will be tested in the next future. Moreover, the pharmaco/toxicological effects of leonurine will also be studied.

DIV P-07

A SPE-HPLC-HRMS method for prohibited performance-enhancing narcotics and stimulants in wastewater for tackling doping via Wastewater-Based Epidemiology

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Background & Aims: Wastewater-based epidemiology (WBE) is an innovative approach for monitoring patterns and trends of illicit drug consumption. The same compounds detected through WBE may also be subject to abuse by amateur and non-amateur athletes across various levels of competition. Therefore, WBE can also have significant applications in the field of anti-doping science, providing a comprehensive overview of performance-enhancing-related activities and offering insights into the broader population's substance misuse trends. Nevertheless, there is currently limited literature investigating the presence of performance-enhancing drugs in wastewater. This study aims at (i) developing a targeted analytical strategy for the detection of suitable biomarkers for prohibited performance-enhancing narcotics and stimulants and their metabolites in wastewater for WBE applications, and (ii) applying the developed method to influent wastewater samples.

Methods: In total, 58 performance-enhancing stimulants and narcotics (9 narcotics and 49 stimulants) were chosen and investigated as potential human biomarkers of drug consumption in sports. These analytes are classified as either S7 Narcotics or S6 Stimulants according to the World Anti-Doping Code international standard prohibited list 2024 and are prohibited for use in competition. A comprehensive review was undertaken to identify potential biomarkers for these drugs for WBE purposes. These performance-enhancing biomarkers were investigated as indicators of misuse for these drugs in wastewater and correction factor values were calculated based on existing urinary excretion data for all compounds. Influent wastewater samples were filtered through glass microfiber filters and spiked with a mixture of internal standards. They were prepared using Bond Elute Plexa PCX (30mg/3mL) Solid Phase Extraction (SPE) cartridges and analysed via high pressure liquid chromatography–high resolution mass spectrometry (HPLC–HRMS) with a run time of 11 minutes. Three experiments were trialled during method development for the SPE protocol, thus evaluating (i) the initial sample acidification step in the SPE process, (ii) different elution solvents, and (iii) different reconstitution solutions.

Results & Discussion: This study developed a targeted analytical method by using SPE-HPLC–HRMS for the analysis of 58 drugs (9 S7 Narcotics and 49 S6 Stimulants) in influent wastewater. The choice to explore S6 and S7 stimulants and narcotics aligns with the broader aim of this study, which seeks to establish WBE as a viable tool for

anti-doping investigations. At pH 7, using a mixture of 3% ammonia in acetonitrile/methanol as the elution solvent demonstrated enhanced extraction efficiencies for both the narcotics and stimulants investigated in this study, compared to pH 2 and using methanol as the elution solvent. The developed method was fully validated in wastewater samples with limits of detection in the range of 0.0125 to 1.0 $\mu g/L$. Recoveries and accuracy were high (> 80% and > 90% on average). The method and instrument precision were on average < 14% and < 13%, respectively. The method was linear for the targeted compounds within the range of 0.025 to 10 $\mu g/L$. Many target drugs, such as methadone, hydromorphone, methylephedrine, were found at quantifiable concentrations in analysed samples.

Conclusion: This method not only enhanced extraction through the improved efficiency of preconcentration and the isolation of analytes but also held significant promise for practical applications in real-world sampling campaigns for tackling doping at community level. This methodology can be seamlessly integrated into comprehensive WBE initiatives, allowing for the robust assessment of the biomarkers identified.

New hemp cultivation policy in Turkiye and worldwide implementations

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DIV P–08

Background & Aims: Hemp has a long history of medicinal and illicit use in many parts of the world. Hemp has been used for thousands of years as a traditional food and as a source of fibre for rope making or textiles. However, the euphoric state it develops due to the psychoactive cannabinoids it contains has led to its abuse and the discovery that it is addictive has led to serious legal regulations.

Recently, industrial hemp (fiber, food, health products, pharmaceuticals, animal feed and building materials from hemp) is seen worldwide as one of the cornerstones in the search for new materials and economic models for a greener and more sustainable economy. In Türkiye, regulations on hemp cultivation have gained momentum with this trend. This study aims to assess the legal regulations on industrial hemp in Türkiye in conjunction with in specific jurisdictions around the world.

Methods: The legal legislations regarding the hemp cultivation and production investigated, within the context of:
I-Hemp cultivation legalizations
II-Definition of Hemp (in terms of allowable THC content)
III-Restrictions
IV- License or authorization in order to cultivate
Sampling Requirements is not included in this study.

Results & Discussion: Hemp Cultivation Legalizations
Türkiye's national legislation on industrial hemp production is based on "Law on the Control of Narcotic Drugs" adopted in 1933. Hemp cultivation for fiber, seeds, stalks and flowers and leaves for the pharmaceutical active ingredients are subject to the permission of the Ministry of Agriculture and Forestry.

Türkiye acceded to the "Single Convention on Narcotic Drugs" in 1966. With this convention, restrictions and controls were imposed on the cultivation of industrial hemp. "Regulation on Hemp Cultivation and Control" in 1990 Türkiye has determined the procedures regarding the determination of the regions where hemp cultivation will be carried out.

On April 5, 2023, the Official Gazette amended the Regulation on Cannabis Cultivation and Control, with this regulation, cultivation has officially started in 20 provinces in our country. Cultivation of hemp for restricted purposes was legalized in Japan in 1947, United States in 2019, United Kingdom in 1993, Canada in 1998, New Zealand in 2006, Russia in 2007, Australia and Italy in 2016, Israel in 2019, Ecuador in 2020, China in 2010 and Thailand in 2021, Colombia in 2022. Malawi, South Africa and Zimbabwe in 2019.

Definition of Hemp (in Terms of Allowable THC content)

In our country, THC level of below 0.3%. Argentina, Australia and Switzerland THC content lower than 1%. In Italy THC level of below 0.6%. Canada, Czech Republic, Austria and Colombia, THC level of below 0.3%. In the UK, Denmark, USA, Germany, THC level of below 0.2%, In Russia, THC level of below 0.1%. Current levels vary across countries and are also changed from time to time.

As is seen in above, THC is the only parameter currently used in industrial hemp cultivation in the world. However, according to recent scientific studies, it has been shown that beside Δ9-THC, Δ8-THC also has a psychoactive effect and even other phytocannabinoids, including CBD, are converted into Δ9-THC. It is important that this information is taken into account in new regulations.

License or authorization in order to cultivate

In Türkiye, if the farmers who will cultivate hemp have previously committed the crime of cultivating hemp without permission, manufacturing, distributing, trading and using cannabis, their applications will not be evaluated according to the Turkish Penal Code and hemp cultivation will not be allowed. Italy does not require a license or permit unless the plants have a THC content above 0.2%. Canada has an online application process with identity and ownership verification requirements, but does not include a criminal background check.

Ecuador and Israel require a background check, while in New Zealand a person convicted of a crime under the country's drug laws cannot obtain a license. The UK has an online application for a license that includes an enhanced background check. In Colombia, a permit is required to grow cannabis. Authorities may carry out control and monitoring visits to locations where cannabis-related activities are carried out to verify compliance and may suspend, revoke or withdraw authorizations.

As industrial hemp cultivation increases in Türkiye, it is inevitable that cannabis containing products (CBD Oil / Hemp Oil) will also increase. Since our current legislation does not distinguish between medical cannabis cultivation and hemp cultivation, it may cause problems in the production and control of these products.

Conclusion: Delta 9-THC is currently the only parameter used in industrial hemp cultivation in the world, but recent scientific studies have shown the presence of other psychoactive cannabinoids. In this context, the rapid increase in the use of cannabis-containing products has increased the number of studies in the field and the importance of re-evaluating the parameters used in exposure assessment.

DIV P–09

Serum and semen PFAS biomonitoring in a highly exposed population: the case of Veneto Region

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Background & Aims: The peculiar heat resistance, lipophobic and hydrophobic properties of per- and polyfluoroalkyl substances (PFAS) led to their widespread diffusion. Specifically, these chemicals are used for waterproof coatings, carpets, leather, and work wear in textile industries, for food packaging, insecticides, firefighting foam and many other applications. Consequently, humans are exposed to PFAS through the ingestion of contaminated food, water and inhalation of airborne particles. Notably, the occupational exposure is the most prevalent route, especially in manufacturing industries. In this regard, there are significant concerns related to the PFAS stability to both chemical and enzymatic degradation, which results in the bioaccumulation in human tissues with a long half-life, estimated of 5 years for perfluorooctanoic acid (PFOA), the most used compound of this class. This accumulation may lead to adverse health conditions, such as increased cholesterol levels, adverse reproductive outcomes, disruptions in hormone regulation, hypertension and obesity.

In 2013, a public health emergency occurred in Veneto Region (Italy), where PFAS contamination was assessed in drinking water and, consequently, in biological matrices of population. The aim of this study was to determine the PFAS concentration in paired serum and semen samples of 524 male volunteers from Veneto Region 10 years after 2013 emergency, in order to monitor their exposure and the disposition in these biological matrices.

Methods: Paired serum and semen samples were collected from 524 male volunteers from Veneto Region, in north-eastern Italy. A total of 17 PFAS were determined by a validated ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) analytical methodology, including perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluoroheptanoic acid (PFHpA), PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUdA), perfluorododecanoic acid (PFDoA), perfluorobutane sulfonic acid (PFBS), perfluoropentane sulfonic acid (PFPeS), perfluorohexane sulfonic acid (PFHxS), perfluoroheptane sulfonic acid (PFHpS), perfluorooctane sulfonic acid (PFOS), perfluoro-2-ethoxyethane-sulfonic acid (PFEESA), perfluoro-4-methoxypropanoic acid (PFMOPrA), perfluoro-4-methoxybutanoic acid (PFMOBA) and the recently synthesized Gen X. For all the analytes under investigation, limit of detection (LOD) was 0.1 ng/mL and limit of quantification (LOQ) was 0.5 ng/mL. Student's paired t-test was performed to evaluate correlations between PFOA concentration in serum and semen samples, since it was the only analyte detected in >50% both matrices.

Results & Discussion: PFOA was the most detected compound, with detection rates of 100% and 94% for serum and semen samples, respectively. More specifically, PFOA was determined in 100% serum samples (median: 28.1 ng/mL; min-max: 0.5-368 ng/mL), while it was quantified in 64% semen samples (median: 1.3 ng/mL; min-max: 0.5-33 ng/mL). Considering the widespread use of this chemical in the industrial area of Veneto Region, a high exposure was predictable; indeed, these values highlighted the bioaccumulation and the persistence of PFOA after 10 years from the

public health emergency. However, the obtained median value was lower than that measured in a recent study considering the same geographical area from 2017 to 2022. The resulting p-value was <0.05, indicating a significant correlation between paired serum and semen concentration. PFOS showed a higher detection rate in serum than in semen; indeed, it was found in 96% serum samples (median: 3.2 ng/mL; min-max: 0.5-41.8 ng/mL), while it was quantified only in 1.5% semen samples (median: 0.8 ng/mL; min-max: 0.5-1.5 ng/mL). Similar results were obtained for PFHxS, which was found in 90% serum samples (median: 2.5 ng/mL; min-max: 0.5-42 ng/mL), while only in 1.7% semen samples (median: 0.6; min-max: 0.5-0.7 ng/mL). Other compounds detected in serum specimens were PFDA (median: 0.6 ng/mL; min-max: 0.5-2.5 ng/mL), PFHpS (median: 0.9 ng/mL; min-max: 0.5-8.0 ng/mL) and PFNA (median: 0.7 ng/mL; min-max: 0.5-1.9 ng/mL) with detection rates of 38%, 26% and 8%, respectively; contrarily, the latter analytes were found in less than 1% semen samples. Finally, PFBA, PFPeA, PFHpA, PFUdA, PFDoA, PFBS, PFPeS, PFEESA, PFMOPrA, PFMOBA and GenX were found in less than 1% both matrices, similar to other studies available in literature.

Conclusion: The obtained results underscored the significant presence of PFAS in the environment and their accumulation in biological matrices. The high detection rates of PFOA highlighted the widespread exposure of individuals in Veneto Region even after 10 years from the public health emergency. For this reason, the continuous monitoring of PFAS concentrations plays a crucial role in understanding the trend of human exposure and to check whether the efforts to address this emergency are presenting effects in the population. Considering the reported adverse health conditions related to PFAS, more studies are needed to describe a comprehensive PFAS impact profile on humans.

Chlorthalidone *in vitro* metabolite identification for documenting exposure in doping

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DIV P–10

Background & Aims: Doping agents are largely illegally used to increase sports performance, acting on strength, muscle mass, and endurance. Among the different substances of this heterogenous group, diuretics can be used both to favor weight loss and mask the intake of another illegal doping agent, altering urine volume and pH and inhibiting the passive excretion of basic and acidic drugs in urine. As a consequence, diuretics are banned by the World Anti Doping Agency (WADA). Diuretics can be divided into several categories according to their mechanism of action, such as carbonic anhydrase inhibitors, Na+/K+/2Cl- symporter inhibitors, osmotic diuretics, renal epithelial Na+ channel inhibitors, mineralocorticoid receptor antagonists, and Na+/Cl- symporter inhibitors. This last group is also known as thiazide-like diuretics, due to the structure of compounds. Chlorthalidone is a WADA-banned thiazide-like diuretic that is used therapeutically to treat disorders such as high blood pressure, renal tubular acidosis, and swelling. Chlorthalidone human metabolic fate is not yet clear, but it is known that it is partially excreted in urine in its parent form. The detection of chlorthalidone metabolites, however, may help find biomarkers to document consumption in doping. This study aims to identify and characterize new specific metabolites of chlorthalidone.

Methods: Chlorthalidone phase I and phase II metabolites were predicted through the open-access software GLORYx (Hamburg University, Germany) to identify the transformations that may occur with higher probability in humans; all compounds with a probability score higher than 25% were used to assist in metabolite identification. Chlorthalidone was incubated with 10-donor-pooled human hepatocytes for 3 h at 37°C. Samples analysis was performed with reversed-phase liquid chromatography coupled with high-resolution tandem mass spectrometry (LC-HRMS/MS) in positive- and negative- ionization modes in full scan and data-dependent MS/MS acquisition modes to record the signal of chlorthalidone and its potential metabolites and their fragmentation pattern in a single analysis. Raw data were processed with Compound Discoverer (Thermo Scientific) with a targeted and non-targeted screening to identify and characterize predicted and non-predicted metabolites.

Results & Discussion: Chlortalidone was detected in both positive- and negative-ionization modes but was more intense after negative ionization. Through GLORYx software, nineteen first- and second- generation metabolites were predicted, the predominant transformation after the preliminary study being N-acetylation and O-glucuronidation, and their combination with sulfation, and glutathione conjugation.

After the analysis of human hepatocytes incubates, several metabolites were identified. The most common transformations were hydroxylation, reduction, glucuronidation, and their combination. The most intense metabolite was found after negative ionization, and it presented a reduction at the tetrahydroisoindolone moiety. Another major metabolite identified in negative-ionization mode presented a hydroxylation at the same part of the molecule.

Conclusion: The use of doping agents in sports has always been and still is a source for concerns. This is precisely why it is essential to provide new biological biomarkers that can identify the intake of these substances. There is well established evidence showing that the metabolism produced in vitro using human hepatocytes effectively mimic in vivo conditions. The present study provides the first overview on chlorthalidone metabolic pattern in humans to demonstrate its consumption in doping.

DIV P–11

Human metabolism of ostarine, a selective androgen receptor modulator and doping agent, with human hepatocytes and authentic positive urine

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Background & Aims: Selective androgen receptor modulators (SARMs) are a family of pharmaceutical drugs that have reached the performance enhancement market during the last decade. These substances are being investigated for possible therapeutic use in hormone replacement treatment and male infertility as tissue-targeting agents. They include substances like ostarine (also known as enobasarm or MK 2866), hydroxyflutamide, bicalutamide, and andarine. In the area of androgen receptor regulation, these benzene-propanamides show great therapeutic potential, and further research is being conducted on their possible applications. Additionally, their lack of androgenic activity suggests that they do not cause the negative effects associated with steroid treatment. Consequently, the World Anti-Doping Agency (WADA) has prohibited SARM use at all times in and out of competition in 2008 under the category "other anabolic agents" (section S1.2 of the WADA list). According to the 2023 WADA report, ostarine is one of the most commonly used substances of this category. The aim of this study was to further investigate the human metabolism of ostarine and identify specific biomarkers of consumption in doping using in silico metabolite predictions, human hepatocyte incubations, and metabolite detection in urine samples.

Methods: Ostarine was incubated for 3 h with 10-donor-pooled human hepatocytes provided by Lonza (% female donors, 50%; % Caucasian donors, 90%; % Asian donors, 10%; age average, 37.0 years, BMI average, 25.0 kg/m2). Incubates were analyzed by LC-HRMS/MS in full-scan MS and data-dependent MS/MS acquisition. Compound Discoverer (Thermo Scientific) mined the raw data. Analyses were supported by in silico metabolite predictions in humans with GLORYx open-access software (Hamburg University, Germany). Metabolites were identified in positive urine samples from autopsy cases following the same LC-HRMS/MS and data mining workflow, with and without glucuronide hydrolysis in urine, to confirm in vitro results.

Results & Discussion: A total of 10 metabolites were detected after 3 h incubation. Seven metabolites were detected in urine samples, and one additional metabolite, generated after combination of ether cleavage and O-glucuronidation, was detected in one urine sample. In vitro and in vivo results were consistent. Consistent with published literature, most intense metabolites were detected in non-hydrolyzed hepatocytes and authentic urine samples after O-glucuronidation. Moreover after enzymatic hydrolysis, hydroxylation was the major biotransformation occurring in real samples. Minor metabolites were also formed after ether cleavage in combination with dealkylation, hydroxylation, or O-glucuronidation.

Conclusion: In vitro and in vivo results were consistent for ostarine, demonstrating the efficacy of the 10-donor-pooled human hepatocyte model to predict human metabolism. We suggest the parent drug and metabolites produced after O-glucuronidation, hydroxylation, and combination thereof as biomarkers of exposure to ostarine in humans. Considering that routine screening of urine samples usually include glucuronide hydrolysis, hydroxylated ostarine and parent drug are proposed as biomarkers of comsuption. Nevertheless, the O-glucuronitated metabolite was the most intense metabolite in non-hydrolyzed samples and therefore represents a valid consumption indicator.

DIV P–12

Genome-wide association study of substance use disorders

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Background & Aims: Nowadays, substance use disorders (SUDs) represent a worldwide public health concern, with huge socioeconomic burden. SUDs are the result of multiple genetic and environmental risk factors; given their multifactorial nature, researching SUDs causes has proven challenging. The synerigistic collaboration between forensic toxicology and genomics can lead to a better understanding of susceptibility factors, helping in diagnosis, treatment, and prevention. The genome-wide association study (GWAS) is a common tool to identify genetic variants associated with complex traits, including psychiatric disorders. The aim of our project is to identify single nucleotide polymorphysms (SNPs) associated with substance use disorders.

Methods: Within a multidisciplinary research project, we performed a genome-wide association study on a sample size of 720 subjects who died in Galicia and in Rome area between 2014-2023 for causes related to substance use, or with a known drug addiction history, or which showed drugs of abuse in their biological samples. Toxicological analyses were carried out by: gas chromatography-mass spectrometry (GC-MS); liquid chromatography coupled to Orbitrap high resolution mass spectrometry (LC-orbitrap-HRMS); high performance liquid chromatography coupled with triple quadrupole mass spectrometry (HPLC–MS/MS). The determination of ethanol was carried out by gas chromatography with flame-ionization detection (CG-FID). Both carbon monoxide CO(carboxyhemoglobin) and cyanide were determined by ultraviolet-visible spectrophotometry UV/Vis. All samples were genotyped with the "Axiom Spanish Biobank" array, which contains 757,836 markers. Controls were taken from the Spanish DNA biobank (https://www.bancoadn.org). A quality control procedure was carried out for the study samples and control datasets. Association testing was computed in Plink, with logistic regression and additive model. The Manhattan Plots (MPs) have been represented in R with the qqman library. Study data were collected and managed using REDCap electronic data capture tools.

Results & Discussion: We found a variant that shows a statistically significant association ($p < 5x10-8$) with SUDs in the SMOC2 gene. This gene in human is involved in embryogenesis, wound healing and angiogenesis. Additional work is needed to determine the relationship of these functions to SUDs or whether the gene has additional biological functions. Alongside, our analyses highlighted variants with $p< 5x10-6$ in genes already known for their function in substance use disorders, supporting the idea of genetic risk factors in SUD. Among these, we highlight LRPPR gene that is associated with smoking initiation and substance use disorders; CNTNAP2 gene, associated with substance use disorders, obsessive compulsive disorder, schizophrenia and depression and GRM5 gene, a promising marker for SUDs as several studies have investigated the role of receptor mGlu5 (encoded by GRM5) in addiction.

Conclusion: The SMOC2 gene does not seem to be related to psychiatric disorders, but this result may be a starting point for other studies. Furthermore, we identified other SNPs that are not statistically significant but are nevertheless interesting because they are found in genes that are already known to have a role in SUDs. The whole of our study strongly supports the assumption that SUD has a strong background of genetic predisposition. Further studies are needed to investigate the genetic pathways and neurobiological mechanisms that underlie the genetic of substances dependence. In the future we plan to perform a meta-analysis with other GWAS and replicate these results in another cohort.

Emerging trend of paraphenylenediamine (PPD) poisoning in Pakistani youth: 10-year analysis

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DIV P–13

Background & Aims: Paraphenylenediamine (PPD) is a chemical compound commonly utilized in hair dyes and henna tattoos. Despite its widespread application in cosmetic items, PPD has garnered infamy as a hazardous substance, notably in instances of intentional self-poisoning. Exposure to PPD can lead to severe health issues such as respiratory difficulties and multi-organ dysfunction. Paraphenylenediamine (PPD) poisoning, known locally as "black stone" in Pakistan, has become a concerning trend, especially among young people, predominantly females. This study aims to elucidate the alarming statistics and demographics associated with PPD poisoning in Pakistani youth, with a primary focus on females. Additionally, the study seeks to interpret forensic toxicology results to ascertain the cause of death in PPD poisoning cases.

Methods: Data were collected from tertiary healthcare hospitals across 11 districts in Pakistan over a ten-year period from 2013 to 2022. A systematic review of 24 articles published in both international and Pakistani medical journals was conducted to compile comprehensive data on PPD poisoning cases. Information regarding patient demographics, clinical presentations, geographic distribution, intentions behind poisoning incidents, mortality rates, and pathological findings was extracted and analyzed. Statistical analysis was performed to identify significant trends and associations within the dataset.

Results & Discussion: A total of 4103 patients of PPD poisoning were reported, 67.5% were females, indicating a disproportionate prevalence among women. The majority of cases (81%) fell within the age range of 20 to 30 years, highlighting the vulnerability of young adults to PPD poisoning. Geographically, South Punjab accounted for 51% of cases, followed by Interior Sindh with 32.5%. Suicide emerged as the primary intention behind PPD poisoning, constituting 87.5% of cases, underlining the urgent need for mental health interventions. The overall mortality rate attributed to PPD poisoning was 29%, signifying its lethal potential. In the second half of the decade (2018-22), only 1100 out of 4103 cases of PPD poisoning were reported, indicating a decrease to 26.5% as compared to the

first half (2013-17) of the decade. However, the mortality rate increased to 32%, and female patients accounted for 68.2% of cases during 2018-22. Recent statistics reveal that two districts of South Punjab, Bahawalpur, and Lodhran, continue to experience a high number of PPD poisoning cases. Cervicofacial edema was the most commonly observed pathological finding (89%), often necessitating tracheostomy in 70% of patients.

Conclusion: PPD, a readily available and inexpensive chemical in local markets of Pakistan, poses a grave threat to public health, particularly among the youth. Urgent regulatory measures are warranted to ban the sale and distribution of PPD as a hair dye constituent. Moreover, the majority of cases hailed from rural and low socio-economic backgrounds, with more than 90% of female patients attempting suicide due to factors such as illiteracy, financial constraints, and social stress. Additionally, concerted efforts from human rights organizations are imperative to address the underlying socioeconomic factors driving suicidal behavior, particularly among young females. Despite a decline in cases in recent years, mortality rates remain high, emphasizing the urgency for regulatory measures and mental health interventions. Toxicologically, attributing a death to PPD poisoning is challenging due to limited literature on its lethal levels. Additionally, interpreting toxicity is complicated by PPD's two-phase toxicity. The first phase, occurring within an hour of ingestion, leads to symptoms like tongue swelling, angioedema and respiratory distress. If death occurs during this phase, PPD is easily detectable. However, in the second phase, patients may initially show signs of recovery before succumbing to complications like rhabdomyolysis and acute renal failure days or weeks later. Detection of PPD in this phase is difficult as it may have been eliminated from the body. Therefore, determining the cause of death relies on any concentration of PPD detected along with histopathological findings of angioedema and acute renal failure. This study contributes valuable insights into the emerging trend of local fatal poisons in developing countries like Pakistan, paving the way for further research, development, and standard guidelines for toxicological analysis and interpretation of poisons like PPD and its metabolites.

DIV P-14

Investigating trends among people who inject drugs: Spatiotemporal analysis of the drug content of used syringes in Sydney, Australia

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Background & Aims: Understanding drug use trends among people who inject drugs (PWID) is frequently based on self-reported data. Whilst insightful, it can be subjective and provides limited information on the drug composition. The chemical analysis of the residual drug content in used syringes has been shown to complement self-reported information. Chemical analysis can confirm the main drug injected, detect other components (e.g. adulterants) and provide an early warning system for emerging new psychoactive substances (NPS) in the drug supply. Injecting drug use is dynamic; hence, longitudinal and geographical analyses might provide greater insight into market behaviours and the preferences of PWID. This study aims to examine the residual drug content of used syringes over time and in different locations in Sydney, Australia, to observe trends in injecting drug use.

Methods: Used syringes were collected throughout four periods from July 2022 until October 2023 from the Medically Supervised Injecting Centre (MSIC) (n=657 syringes). Specimens were also collected from disposal facilities adjacent to four needle and syringe program (NSP) locations in Sydney across one sampling period in October 2023 (n=888 syringes). The residual content was extracted with methanol, filtered and spiked with phenazine before detection and semi-quantification by gas chromatography - mass spectrometry (GC-MS) and ultra-performance liquid chromatography – tandem mass spectrometry (UPLC-MS/MS); the chemical results from MSIC syringes were aggregated and compared to the drugs self-reported by MSIC clients (using Kendall Rank Correlation). The different samples were collated to conduct spatial and temporal investigations of injecting drug and market trends in Sydney.

Results & Discussion: Heroin, methamphetamine and pharmaceutical opioids (namely methadone) were the most frequent drugs detected, with minor fluctuation between samples. The correlation between drugs chemically detected and those self-reported was positive. Adulteration and polydrug mixtures remained low throughout all samples. Heroin was the most common drug to be found in the presence of other drugs/adulterants; polydrug mixtures with methamphetamine were the most common. Although not frequent, instances of concern involved polydrug mixtures, specifically singular occurrences of the following combinations with heroin: 2'-fluoro-2-oxo PCE (ketamine NPS), α-pyrrolidinopentiophenone (cathinone NPS), and fentanyl (in separate syringes). There were geographical differences across sampling sites related to the most common drug injected (varying between heroin and methamphetamine); adulteration occurred more frequently at disposal facilities adjacent to NSP locations (e.g. at one disposal facility, 50% of heroin syringes contained caffeine, compared to 4% of heroin syringes collected from MSIC across all time periods).

Conclusion: Drug residues from syringes collected across Sydney were examined, and detections of heroin, methamphetamine and pharmaceutical opioids were relatively stable over the period examined. Some differences were observed across sampling sites highlighting different market characteristics and use among different communities. This research holds great potential for harm reduction for both individual (i.e. PWID) and organisational levels by providing insights into the current drug market.

Blood lead levels (bll) in lead acid battery industries: A comprehensive study

DIV P–15

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Background & Aims: Lead exposure poses a significant global health concern due to its irreversible effects. Short-term exposure to high doses (BLL ≥ 60 µg/dL) can result in anaemia, weakness, and damage to the kidneys and brain. Chronic exposure to low doses (10 – 40 µg/dL) may lead to joint pain, heart disease, tremors, numbness, and decreased bone density. Workers in industries involving lead face heightened exposure risks [1]. There has been no study conducted in Uganda to assess Blood Lead Levels (BLL) among workers in the Lead Acid Battery industry. This study aims to investigate BLL levels, potential causes of exposure, available controls, and work practices. Additionally, it includes qualitative respirator fit testing to determine if poorly fitting respirators contribute to elevated Blood Lead levels.

Methods: The study began with a health assessment (lead related) questionnaire for workers, followed by blood sampling. Work practices and engineering controls were also assessed. In addition, qualitative respirator fit testing was performed. Blood collection and analysis followed NIOSH method No. 8003 on an Agilent 200 Series Graphite Furnace Atomic Absorption Spectrometer (LOD = 0.3 µg/dL, LOQ = 1 µg/dL). Respirator fit testing adhered to OSHA Standard 29 CFR 1910.134 Appendix A [2].

Results & Discussion: In total, 112 workers (107 males and 5 females) participated in this study. From the results, 13% (n=15) of the workers had undetectable levels of BLL, the rest 87% (n=97) had detectable BLL. From these, 64.3% (n=72), had BLL ranging between 1 - 19 µg/dL, while 22.3% (n=25) of the workers had BLL equal to or exceeding the Biological Exposure Index (BEI) of 20 µg/dL, as established by the American Conference of Governmental Industrial Hygienists (ACGIH). The workers had an overall average BLL of 13.84±11.25 µg/dL.

From the questionnaire, at least 40% (n=45) of the workers experienced one or more symptoms related to lead exposure such as headaches, joint pains, general body weakness, gastrointestinal challenges, itchy skin and itchy eyes.

From the respirator fit testing exercise, 24% (n=5) passed the Fit Test. The rest of the workers 76% (n=16), failed the respirator fit testing at different stages of the test.

Conclusion: The elevated BLL in workers was due to the fact that workers were not well protected in terms of engineering controls, poor fitting respirators and very poor work practices, which included eating and drinking without washing their hands or faces.

Characterization of serotonin transporter modulators employing a new *in vitro* activity-based assay

DIV P–16

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Background & Aims: The human serotonin transporter (hSERT; SLC6A4) is a pertinent target for a wide range of psychiatric therapeutics due to their role in maintaining serotonin (5-HT) homeostasis. Importantly, it is also targeted by many illicit drugs and new psychoactive substances (NPS), which may disturb 5-HT homeostasis by inhibiting or reversing SERT activity. The dangers associated with stimulant NPS use, potentially causing life-threatening situations, have resulted in an increased interest in how (and what aspect of) these substances modulate this transporter. Current methods to assess SERT activity monitor the uptake of radiolabeled or fluorescent substrates. Although these approaches can provide relevant information, they pose limitations to either throughput and/or the use of a non-endogenous substrate mimicking the 5-HT uptake event. In this study, a new in vitro activity-based assay was developed to assess a compound's impact on SERT. The principle of the new assay format, coined 'TRACT', 'TRansporter ACtivity Through receptor activation', relies on the communal involvement of 5-HT in both reuptake by SERT and activation of the 5-HT receptor (5-HT2AR), a G protein-coupled receptor (GPCR). More specifically, the new approach combines SERT and the 5-HT2AR in one cell system and assesses to what extent 5-HT, which is supplied to the cells, is able to activate the 5-HT2AR. In the absence of SERT inhibition, 5-HT will be rapidly transported inside the cell and will not (or hardly) be able to activate the 5-HT2AR. In contrast, in the presence of SERT inhibition, 5-HT will remain extracellular, and

will be able to activate the 5-HT2AR. Additionally, to gain a comprehensive understanding of the specific mechanism of action of the tested compounds, the compounds were also evaluated for their (potential) direct interaction with 5-HT2AR, in cells only expressing this receptor (in the absence of SERT expression).

Methods: The TRACT assay principle was developed starting from a stable 5-HT2AR-expressing human embryonic kidney (HEK293T) cell line, that was transfected with a hSERT-encoding plasmid. The latter will efficiently pump extracellularly administered 5-HT inside the cell, thus largely precluding 5-HT2AR activation. 5-HT2AR activation is monitored via a split-luciferase system (NanoBiT®, Promega), in which activation of 5-HT2AR, fused to one subunit of a nanoluciferase enzyme (NLuc), initializes the recruitment of β-arrestin2, fused to the other subunit, allowing reassociation of the two subunits and restoration of a functional NLuc. After addition of the substrate furimazine, a strong bioluminescent signal is generated and used as read-out. When SERT activity is blocked, this results in an increased extracellular presence of 5-HT, and, as a result, increased 5-HT2AR activation. Different assay parameters were evaluated and optimized using paroxetine as reference SERT inhibitor: amount transfected SERT DNA, incubation time with the substance prior to activation with 5-HT and the concentration of 5-HT. Applicability of the optimized assay format was demonstrated using three different classes of antidepressants (SSRI, SNRI & TCA) and one cathinone, naphyrone that has a high potency for SERT inhibition, and the results were compared with those obtained using a commercially available fluorescence-based assay (Molecular Devices®). To elucidate a compound's (potential) direct impact at the 5-HT2AR, selected compounds were evaluated for their agonist or inverse agonist activity at 5-HT2AR, as well as for their ability to compete with 5-HT for 5-HT2AR binding (antagonism).

Results & Discussion: The optimal assay format involved 1.65 µg transfected SERT DNA per well (6-well plate) and 30 min incubation with the substance prior to the addition of 100 nM 5-HT. The SERT inhibition ranking order of the antidepressants and naphyrone, obtained by the TRACT assay, largely mirrored that of the fluorescence-based assay, demonstrating its applicability. Moreover, the TRACT assay successfully identified compounds interacting with the 5-HT2AR, exhibiting either antagonism or inverse agonism at the 5-HT2AR.

Conclusion: The novel in vitro activity-based assay was demonstrated to be suitable for the characterization of SERT modulators using interconnected 5-HT2AR activation as read-out. Besides monitoring SERT inhibition, the TRACT assay was also capable of identifying a compound's intrinsic action at the 5-HT2AR: agonism, antagonism and inverse agonism. The universal character of the TRACT assay allows the employment of only one assay to gain insight into a compound's (different) mechanism of action(s) at both SERT and 5-HT2AR.

DIV P-17

Driving under the influence of ketamine: The Universitary Center of Trafic Medicine Lausanne - Geneva experience

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Background & Aims: Any psychoactive substance acting on the central nervous system can impair driving skills. Driving is a complex task that places demand on vision, rapid decision making, planning, tracking, vigilance, reaction time, coordination, and gross motor activity. Most importantly, driving requires the ability to divide attention between several competing demands on cognitive skill. Any of these functions can be adversely affected by psychoactive substances either alone or in combination. Misuse of dissociative drugs is a popular phenomenon and a cause for clinical concern. Among these, ketamine misuse as a recreational drug has remarkably increased over the last decade and is now being reported from many different countries. Ketamine is especially popular among youngsters and is self-administered either alone or in combination with other drugs. Whereas acute psychotropic effects of ketamine may cause discomfort for some individuals, its dissociative properties have made it desirable for recreational use. Users report that lower doses induce mild stimulatory, dissociative, and hallucinogenic effects, whereas higher doses yield psychotomimetic symptoms and separation from reality. The most common route of recreational administration is nasal insufflation, with an onset of feeling "high" ranging between 5 and 10 minutes and lasting between 40 and 75 minutes. Ketamine has been shown to cause alterations in eye movement, decreased visual search performance, and increased time spent off-target. Ketamine users may have significant difficulty tracking the road and other objects moving on the road, including people or other cars, thus making users more at risk of causing road accidents or being involved in them.

The objective of this study is to outline the characteristichs of cases of driving under the influence of ketamine that were observed in our faclity as part of a medical assessment of fitness to drive over a period of two years.

Methods: 28 cases of drivers who were stopped by the police due to driving under the influence of ketamine during the période September 2021 and August 2023 and who contacted our facility in order to undergo a fitness-to-drive medical assessment in the period between January 2022 and December 2023 were retrospectively reviewed. In all cases, ketamine finding was confirmed by toxicological investigations.

Results & Discussion: Ketamine users who were examined in our facility were in the 18-28 age group, with a male prevalence. In the majority of cases, they were poly-users of illicit drugs, with previous ketamine consumption for at least one year.

All individuals described recreational ketamine use exclusively.

The most frequently described researched effets were out-of-body experience, depersonalisation and euphoria, which is consistent with data described in the literature.

None of the subjects reported visual field narrowing following ketamine use. On the other hand, some of them recognised a possible increase in impulsive behaviour while driving.

Generally speaking, ketamine users may have significant difficulty tracking the road and other objects moving on the road, including people or other cars, thus making them more at risk of causing road accidents .

Long-term use of ketamine can lead to tolerance, dependence, withdrawal signs, and flashbacks with symptoms and perceptual distortions that may persist even after the individual is no longer using ketamine.

Conclusion: Considering the long-term effects of prolonged consumption of ketamine as well as multiple illicit substances with psychotropic effects, the possibility of using routine neuropsychological investigations should be taken into consideration during fitness-to-drive medical assessment in ketamine users.

Comparison of concentrations of common psychotropic and substitute drugs in serum stabilised with and without sodium fluoride

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Background & Aims: For clinical or forensic toxicological examinations, blood is the preferred matrix. In many cases, however, plasma or serum (obtained by centrifugation) is routinely used for this purpose. reagents, such as sodium fluoride, are useful in preventing drug degradation in collected blood samples, helping to avoid adulteration of drug analysis results. The aim of the study was to examine the potential impact of sodium fluoride addition on serum concentrations of various prescribed psychotropic drugs.

Methods: Patients (n = 92) were recruited at the Department for substance dependence and psychotherapy of the LVR Hospital in Bonn (Germany). Data was obtained on medication, age, and sex of the participants. In total, 100 paired blood samples were collected using serum tube devices with and without sodium fluoride addition (50 mg). The collected blood samples were immediately centrifuged at 1250 x g for 5 min. The resulting supernatants with and without fluoride were transferred to separate cryotubes. Routine liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods were used to analyse serum samples for different neuroleptics, antidepressants, benzodiazepines, opioids, and anticonvulsants. The concentration ratio of each analyte was determined between fluoridated and non-fluoridated serum samples. A paired two-tailed t-test ($\alpha = 0.05$) was conducted to statistically evaluate the quantitative results of each compound in serum with and without previous fluoride addition. The study design was approved by the ethics committee of the Rhenish Friedrich Wilhelm University of Bonn.

Results & Discussion: After centrifugation, a noticeable difference was visible between serum samples stabilised with fluoride and those without previous fluoride addition. The supernatants that were stabilised with fluoride showed a reddish colour, while the supernatants that were not stabilised with fluoride showed a clear to yellowish turbidity. Median concentration ratios (fluoride-stabilised/fluoride-free) for most compounds ranged from 0.73 to 0.93, indicating that samples obtained from blood previously stabilised with fluoride have on average 7% to 27% lower concentrations than samples without fluoride. Only the active Venlafaxine metabolite O-desmethylvenlafaxine revealed a median concentration ratio exceeding 1.0 (1.13; +13%). The results were statistically significant ($\alpha = 0.05$) for olanzapine, risperidone, paliperidone, pipamperone, prothipendyl, mirtazapine, N-desmethylmirtazapine, sertraline, quetiapine, venlafaxine, O-desmethylvenlafaxine, clonazepam and 7-aminoclonazepam. Fluoride can promote haemolysis by depleting adenosine triphosphate (ATP) of erythrocytes and causing potassium leakage from the cell. Therefore, haemolysis can cause a shift in red blood cell components and dilution of the serum. The analyte concentrations in fluoride-stabilised serum were lower than those in fluoride-free serum, probably due to dilution of the serum by fluoride-induced haemolysis. This was observed for most of the substances. On the other hand, the produced shift in red blood cell components in fluoride-stabilised samples may have caused a false elevated concentration of O-desmethylvenlafaxine. Overall, it is important to consider haemolysis when results of specimen with and without fluoride are compared as it affects test results and their interpretation.

Conclusion: An accurate interpretation of drug levels for therapeutic drug monitoring or assessment of acute drug-induced impairment depends heavily on the knowledge of the sampling device and/or matrix used for analysis, as for instance stabilising agents such as sodium fluoride may affect drug concentrations.

DIV P–19

"False positive" and "false negative" workplace drug screening test rates in Australia

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Background & Aims: AusHealth performs approximately 70,000 workplace drug tests (testing urine and oral fluid) Australia-wide each year using immunoassay-based devices. Specimens that are 'non-negative' and a proportion of negative specimens selected for QC validation are despatched to a laboratory for confirmation by LC-MS analysis. Confirmed positive specimens render the donor liable for workplace disciplinary procedures. The rate of inaccurate screening results must be kept to a minimum to ensure workers are not needlessly stood down from duties.

The aim of this study was to determine the rates of inaccurate screening test outcomes in a large dataset.

Methods: Data were collated representing 5 years of oral fluid and urine drug test results (approximately 350,000 tests overall). Specimens were screened using immunoassay-based test devices with cut-off concentrations for each drug class shown. Laborator confirmation cut-off concentrations are shown in italics.

Urine: Amphetamine-type stimulants (300µg/L; 150µg/L), Opiates (300µg/L; 150µg/L), Cocaine (300µg/L; 150µg/L), THC-cooh (50µg/L; 15µg/L), Benzodiazepines (200µg/L; 200/100µg/L).

Oral fluid: Amphetamine-type stimulants (50µg/L; 25µg/L), Opiates (50µg/L; 25µg/L), Cocaine (50µg/L; 25µg/L), THC-cooh (15µg/L; 5µg/L), Benzodiazepines (10µg/L; 2µg/L).

Data were sorted to isolate specimens for which both screening and confirmation were performed. True negatives required both screening and confirmation to be negative (below cut-off) and true positives yielded both screening and confirmation positive, "False positives" yielded a screening result of non-negative and a confirmatory negative, and "False negatives" yielded the opposite.

Data for each drug class were entered into a 2x2 table to calculate accuracy, sensitivity, specificity, positive and negative likelihood ratio, positive drug prevalence as well as positive and negative predictive value. Analysis was repeated after examining all confirmation LC-MS data to identify those specimens reported as negative (below cut-off concentration) but with traces of drug present above the limit of detection (LOD).

Results & Discussion: Raw data representing approximately 56,000 urine specimens and136,000 oral fluid between October 2020 and March 2024 yielded the following outputs.

Urine: Sensitivity 82.7%; Specificity 99.9%; Positive Predictive Value 96.9%; Negative Predictive Value 99.1%; Accuracy 99.0%.
Correcting for true positive above LOD (rather than cut-off concentration) changes accuracy to 99.3%.

Oral Fluid: Sensitivity 90.6%; Specificity 85.4%; Positive Predictive Value 71.3%; Negative Predictive Value 95.8%; Accuracy 86.9%.

Correcting for true positive above LOD (rather than cut-off concentration) changes accuracy to 92.3%.

These data demonstrate the utility of oral fluid and urine screening devices as accurate and appropriate to minimise the rates of false positives and false negatives, indicating their appropriateness for reliable workplace drug testing. In addition, scrutiny of aberrant data can reveal the true performance of devices.

Conclusion: Workplace oral fluid and urine drug screening provides reliable and accurate rationale for subjecting specimens to confirmatory analysis that in turn provides good quality evidence for employers to apply sanctions and disciplinary procedures to workers identified as drug positive.

DIV P–20

Drug Facilitated Crimes without an other crime: Filling the gap in victim protection

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Background & Aims: In a drug facilitated crime (DFC), a psychoactive substance is administered to a victim in order to lower their inhibition or render them unconscious, thereby facilitating the commission of a crime such as sexual

assault or robbery. Victims of such crimes are typically well supported by the system, which is set up to collect toxicological evidence of the crime.

In some cases however, while the administration of the psychoactive substance occurs, the victim manages to reach safety prior to the commission of the crime. For example, following a drink spiking or needle spiking occurrence, feeling the effects of the psychoactive substance, the victim requires the assistance of friends to get back home.

While this still constitutes a crime in most jurisdictions, e.g., administration of a noxious substance (ANS) in Canada, victims often fall in a procedural gap where there are no clear instructions for evidence collection.

In December 2023, a new program was instituted in the province of Québec (Canada) to fill this gap and offer answers to individuals who believe they have been drugged without their knowledge. By going to any hospital emergency department, they will have access to urine specimen collection kits. Police officers will send the urine samples collected to the forensic toxicology laboratory for analysis and will initiate an inquiry into the ANS event.

This work will outline the results of the first four months of operation of this program.

Methods: Urine samples collected under the ANS program must come with an adequately filled consent form for toxicological analyses to be performed. Samples collected within 24 hours of the alleged drug administration event will be submitted to two analytical methods: volatiles and broad scope targeted screening, while only the latter will be performed on samples collected more than 24 hours after the event.

For the targeted screening, samples are extracted by protein precipitation and analyzed by LC-MS/MS (Sciex 5500 QTrap). A total of 144 analytes, including GHB, opioids, benzodiazepines, cannabinoids, new psychoactive substances and other drugs are detected. Volatiles analysis is carried out by GC-HS/FID (Agilent) and detects substances such as ethanol, methanol and acetone.

In some rare instances, a GC-MS general unknown screening is also performed. This might be deemed necessary by the forensic toxicologist if, for example, tablets were given to the victim but no drugs were detected in the targeted screening. The urine sample would undergo solid phase extraction (Oasis HLB cartridge) prior to analysis by GC-MS (Agilent).

Positive findings are reported as "detected" in the final report.

Cases are compiled in a database which includes toxicological results, event location (pulic/private, city), event to sample collection delay and gender.

Results & Discussion: Between December 1st 2023 and March 31st 2024 (4 months), 120 ANS cases were received by the forensic toxicology laboratory for a population of 8.9 million. As a comparison, this is about one third of the number of SA cases handled by the department for the same period. From these, 112 were analyzed; whereas eight cases did not undergo toxicological analysis due to the absence of proper consent.

Of the 112 ANS cases analyzed, 88% were positive for at least one substance (psychoactive or not). Ethanol was found in 52% of cases analyzed for alcohol, and it was the only substance found in 23% of cases. These figures are similar to those found in sexual assault cases.

Drugs were found in 65% of cases. Interestingly, GHB, often characterized as a "date-rape drug" commonly used in DFSA, was not found above endogenous levels in any urine sample collected under the ANS program.

Despite participants to this program seeking help because they thought they displayed symptoms of intoxication by a psychoactive substance, 8% of all samples contained no detectable analyte. This could be attributable to complete elimination from the system prior to sample collection. However, most samples (83%) were collected within 20 hours of the alleged ANS event, which should be within the window of detection in urine for most substances. The possibility of alcohol-only consumption should not be overlooked.

Naturally, toxicological analyses cannot differentiate between drugs taken voluntarily and involuntarily. Therefore, it is the responsibility of police investigators to determine whether to proceed with ANS accusations or whether the substances found were voluntarily ingested by the victim.

In ANS as in SA cases, victims are overwhelmingly female (87% vs 96%). Unsurprisingly, ANS events occur in a public location much more frequently than SA (70% vs 15%).

Conclusion: The high number of ANS cases sent for analysis confirm the relevance of such a service, which will likely increase in popularity as it becomes more widely known. In the data compiled thus far, ethanol remains the most prevalent substance, as is the case in SA cases handled by the laboratory. As months go by, additional ANS data will be compiled, completing the picture drawn here.

DIV P–21

Pharmacokinetic assessment of clozapine concentration-to-dose ratio in patients with mental disorders: Implications for therapeutic drug monitoring

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Background & Aims: Clozapine, an atypical antipsychotic, is utilized in the management of schizophrenia, schizoaffective disorder, and bipolar disorder, typically as a secondary intervention following treatment resistance. The exact mechanism of action of clozapine is not completely understood but clozapine is linked to many serious adverse effects. Therapeutic drug monitoring (TDM) is used to improve patient response and to decrease the risk of adverse effects. Therapeutic reference range for clozapine serum concentration is narrow, 350-600 ng/mL. As clozapine dosage is different depending on diagnosis, age, smoking status, additional therapy, monitoring patient adherence with serum clozapine concentrations is not informative enough. However, the concentration-to-dose ratio (C/D ratio) presents a potentially valuable metric for evaluating pharmacokinetic dynamics, especially considering the linear relationship between clozapine doses and its serum concentrations higher than 150 ng/mL. Furthermore, clozapine C/D ratio is different depending on ancestry, sex and smoking status.

The aim of our study was to determine clozapine C/D ratio in patients with mental disorders among inpatients in our hospital, providing insights into the pharmacokinetic profile and its implications for TDM.

Methods: The study included 50 consecutive patients on clozapine therapy, diagnosed with mental disorders from the Clinic of Psychiatry, Sestre milosrdnice University Hospital Center, Zagreb. Serum samples were drawn at steady state (after 5-7 days of clozapine use without dosage change) and through drug concentrations were determined (prior to the next dose). VACUETTE® TUBE, 4mL, Z No Additive (Greiner Bio-One, Kremsmünster, Austria) were used for sampling. Immediately after sampling, the samples were aliquoted, stored at -20°C and analyzed within 30 days. Serum drug concentrations were determined by liquid chromatography-mass spectrometry on an LCMS-8050 analyzer (Shimadzu, Kyoto, Japan) using the commercial kit: ClinMass Add-On Set for Neuroleptics (Recipe, Munich, Germany). Patients with serum clozapine concentration higher than 100 ng/mL were included as studies report clozapine follows linear kinetics from around 150 ng/ml. C/D ratio was calculated by dividing the clozapine concentration by the daily dose. Normality was tested using the Kolmogorov-Smirnov test. Statistically significant differences were tested using the t-test. Variables are presented as mean ± SD or as median and interquartile range. Statistical analysis was performed using MedCalc® statistical software version 20.008 (MedCalc software, Ostend, Belgium).

Results & Discussion: 32 male, median age 31 years (range 19 to 65 years) and 18 female, median age 43 years (range 20 to 76 years) patients were included in our study. Clozapine median concentration was 182.9 µg/L (141.9 to 272.5). The median administered dose was 200 mg per day. Median clozapine C/D ratio was 1.37 µg/L per mg/d (0.83 to 1.73) among all patients. Mean clozapine C/D ratio in male and female subgroups were 1.19 ± 0.61 µg/L per mg/d and 1.69 ± 0.54 µg/L per mg/d; p=0.005, respectively. 10 patients were smokers, their mean clozapine C/D ratio was 1.34 ± 0.73 µg/L per mg/d and similar median clozapine C/D ratio was among nonsmokers 1.38 ± 0.61; p=0.869. Clozapine C/D ratio in our study is similar as in the guideline on clozapine titration published by de Leon et al. After stratification by sex median clozapine C/D ratios were higher in female subgroups. Although a lower C/D ratio is expected among smokers this was not confirmed in our study. Major limitation of our study is the small number of patients included, and this may be a reason for unexpectedly high clozapine C/D ratio in smokers.

Conclusion: Although the median of serum clozapine concentration in our study is below the lower limit of therapeutic range, C/D ratio indicates that patients were taking therapy properly. Also, higher C/D ratio among female subgroups indicates lower clozapine doses are needed to achieve therapeutic range. So potentially, C/D ratio could be a good parameter for monitoring patient adherence and pharmacokinetic abnormalities.

DIV P–22

Nature is rich: types of (il)legal samples seized at spiritual retreat centers

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Background & Aims: Samples seized in two spiritual retreat centers - suspected of massive use of illegal substances - were submitted to the laboratory for identification. From both places, we received several samples of powder with the indication "Rapé" and several samples of a thin dark brown liquid, in various packaging, sometimes labeled as "Patina vegetal". Moreover, capsules and diverse vegetal samples in raw form, such as leaves, were also seized.

Methods: After dilutions, samples were submitted to a chromatographic analysis by ultra-high-performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-TOF-MS, TripleTof® 4600 from Sciex). No quantification was performed as the simple presence of a controlled compound in a sample allows for its classification as illegal. The raw plant samples were identified based on their macroscopic and microscopic characteristics.

Results & Discussion: Even when they were labeled as "Patina vegetal" (a misleading label is a common scheme to divert attention from the authorities), chromatographic analysis of the brown liquids revealed the presence of dimethyltryptamine (DMT), 5-OH-DMT, harmine, harmaline, and tetrahydroharmine, classical alkaloids found in Ayahuasca. This last is a psychotropic beverage made of plants found in South America: the liana Banisteriopsis caapi, sometimes in combination with other plants, most commonly Psychotria viridi. 5-OH-DMT was also found in the capsules. Next to its psychotropic effect, Ayahuasca also exerts a purgative effect leading to vomiting and diarrhoea that can be impressive.

The other samples common to both seizures were grey-brown powders labeled "Rapé" (Rapé Yawanawa , Rapé Bobinsana,...). Analysis revealed the presence of nicotine in these samples. Indeed, "rapé" are preparations made of tobacco powder mixed with other plants that are insufflated in the nose during rituals. In this way, "Rapé" is supposed to have purifying, energizing and therapeutic properties.

Ibogaine and mescaline were respectively identified in a capsule and a powder. Both share psychotropic properties. These are controlled substances in our country, even if they are very unusual locally.

Finally, untransformed vegetal samples were identified as senna leaves and pods (laxative properties), boldo (can relieve various gastrointestinal disorders), white sage (calmative) and Palo Santo (incense).

Conclusion: Samples were successfully identified and represent a range of available plants intended for "purification of soul and body". Perhaps more widespread in other parts of the world such as South America, these botanicals are not traditional in Europe. In fact, except for cannabis, plant-based samples are rarely submitted for analysis in our lab, representing a small challenge.

The Oregon experience, fentanyl, decriminalization and decriminalization undone; and why it matters

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Background & Aims: This paper considers how the U.S. federal system wherein federal and state authorities have concurrent jurisdiction over controlled substance crimes, have impacted the ongoing fentanyl crisis in the U.S. and consequently the practice of forensic toxicology.

Methods: We review the legislative history of drug decriminalization, lessons learned, and subsequent recriminalization, in the US State of Oregon and its impacts on drug use and the corresponding impacts on fentanyl mortality, impaired driving and consequences for forensic toxiclogy and pathology resources.

Results & Discussion: U.S. federalism has resulted in a patchwork of laws relating to drug decriminalization. The

decriminalization movement began with the proliferation of medical marijuana in California and Colorado in 2008-2009, and the decriminalization of recreational marijuana in many states beginning in 2012. Marijuana is a Schedule I Controlled Substance under the U.S. Code, indicating high abuse potential with no accepted medical use. Despite the federal Schedule I classification, twenty-one states have fully decriminalized marijuana and thirty-eight states allow medical use.

In 2020, the State of Oregon passed the Drug Addiction Treatment and Recovery Act (Measure 110). a voter-approved initiative, which decriminalized possession amounts of all controlled substances including fentanyl, novel opioids, hallucinogens, methamphetamine, psilocybin, cocaine, nitazenes and other NPS. Much has changed in the U.S. since the marijuana legalization movement started in 2012, including the drug overdose deaths of at least 950,000 persons. Yet, Oregon and several other states and cities in the U.S. continue to decriminalize drugs. In Oregon, decriminalization Measure 110 converted possession of any controlled substance from a misdemeanor criminal offense potentially punishable by jail and a fine, to a Class E civil violation where a waivable fine is imposed conditioned on user contact at a health assessment center. Measure 110 pledged increased resources for expanded treatment and services, enhanced community access to health care, and health-based approach to drug addiction and overdose rather than "making criminals out of persons because they suffer from addiction."

Conflicting information abounds regarding the successes and failures associated with Oregon's decriminalization. A study in JAMA Psychiatry found no evidence of association between legal changes that removed criminal penal-

ties for drug possession and fatal overdose rates. CDC data from October 2023 found, however, that drug overdose deaths rose almost 50 percent in Oregon, from 1,171 in 2021 to 1,683 in 2022. In Portland Oregon, officials reported the number of overdose deaths involving fentanyl increased by 533% between 2018 and 2022, compared to national average increase of 52% over the same time period, thereby severely burdening forensic pathology and toxicology services. Forensic toxicology resources were also impacted in driving-related crimes, as the number of DRE evaluations of impaired drivers increased from 264 to 436 between 2018 and 2022 (64%). The Oregon Department of Transportation conducted a 27-county survey (including Multnomah County where Portland is located), and concluded 65% of all driving-related fatalities were substance-involved crashes, and 23% of all fatal and serious bodily injury crashes were substance-involved. Exact substances causing driving impairment are not regularly identified as law enforcement often does not test for other substances if alcohol impairment is evident. The forensic pathology and toxicology workload shortage in Oregon is so severe that the Oregon legislature passed a 2024 bill mandating the formulation of a workforce shortage remediation plan.

As 2024 dawned, the morbidity, mortality, and degradation of cleanliness, safety, and hygiene in cities and the State of Oregon caused a societal shift in attitude. By January 2024, state and city officials declared a state of public emergency in the City of Portland due to the open and notorious use of controlled substances. The Governor of Oregon concluded immediate action was necessary because Oregon was suffering economic and reputational harm from the ongoing fentanyl crisis.

On April 1, 2024, Oregon's Governor signed House Bill 4002 into law. HB 4002, which lawmakers in Oregon overwhelmingly passed in bi-partisan fashion, and which rolled back Ballot Initiative 110's decriminalization efforts. Under HB 4002, possession amounts of fentanyl, heroin, methamphetamine, cocaine, LSD and other substances return to misdemeanor status. Misdemeanors are potentially punishable by jail time, but misdemeanors are also eligible for deflection from prosecution, treatment, and supervision. It is estimated that 1333 more misdemeanor arrest per year will occur post-Bill 4002, although arrest estimates are not valid predictors of future convictions or jail time due to 4002's deflection provisions.

Conclusion: While Oregon and other states experimented with drug decriminalization, in 2023 over 105,000 persons died of overdose deaths in the U.S. Between 10.1.2023 and 2.29.2024, US Customs and Border Protection seized 8,447 pounds of fentanyl. Morbidity, mortality and homelessness rose. These experiences should be taken into account as other states contemplate decriminalization of possession amounts of controlled substances, until root cause analyses of Oregon's experience, and its impacts on fentanyl demand and supply, and its associated harms and impacts on forensic resources are taken into account.

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Investigation of adulterants/diluents and impurities in street heroin using gas chromatography-mass spectrometry and principal component analysis.

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Background & Aims: In the field of forensic drug analysis and investigation, the profiling or chemical fingerprinting of seized illicit drugs is essential. This process involves identifying, quantifying, and categorizing drug samples into groups, which can provide investigative leads such as determining common or different origins of seized samples. Moreover, drug profiling aims to uncover synthetic pathways, detect adulterants and impurities, pinpoint a drug's geographic origin and distribution routes.

Heroin is highly addictive, and its anesthetic effect is ten times greater than that of morphine. Due to its toxicity and potential for addiction, heroin is classified as a Schedule I substance and is highly controlled and illegal. Illicit street heroin is often sold and used in an impure state, with other substances added to increase its bulk quantity and profitability. These added substances are referred to as adulterants or diluents, and they can be toxic in addition to the inherent toxicity of the drug itself.

Methods: The paper presents an analysis of impurities and adulterants in street heroin samples seized in Pakistan and grouping the samples into groups to determine the origin. The study analyzed a total of 519 samples for the presence of cutting agents and impurities in illicit heroin using gas chromatography-mass spectrometry (GC/MS) coupled with in-house built search library and principal component analysis to identify samples of similar chemical profile.

Results & Discussion: The results showed that the street heroin samples contained a range of adulterants and impurities. Street heroin was adulterated with acetaminophen, caffeine, chloroquine, clotrimazole, diazepam, dextromethorphan, methacetin, mephobarbital, nicotinamide, and phenobarbital. The GC/MS results identified several impurities in heroin samples, including acetylcodeine, MAM, codeine, morphine, meconin, papaverine, and noscapine. PCA analysis revealed two distinct clusters in a score plot, indicating the potential for grouping the sam-

ples into two distinct categories. The two distinct groups in PCA reveal the illicit powder in local drug market have two different sources. As, the heroin powder have different sources, in order to control the prevalence and drug trafficking this would be quite helpful. The prevalence of heroin can be checked be from seizure data of the country, with an average of 5414 kilograms of heroin seized by law enforcement authorities over a five-year period from 2017 to 2022. Morphine and opium, two key precursors used in heroin synthesis, were also frequently confiscated.

Conclusion: In conclusion, the results of GC/MS and PCA analysis based on the diverse range of adulterants and impurities underscores the complex nature of the illicit drug market and this study offers crucial insights into the composition and origins of illicit heroin circulating in the country. The identification of distinct groups through principal component analysis suggests the potential for categorizing seized samples, aiding law enforcement efforts in understanding trafficking patterns and targeting illicit drug networks. Furthermore, the consistently high volume of heroin seizures, along with the confiscation of key precursor substances, highlights the ongoing challenge posed by heroin production and trafficking in the country. This research underscores the importance of continued vigilance and collaboration between forensic scientists, law enforcement agencies, and policymakers to address the multifaceted issues surrounding heroin abuse and trafficking.

Development of a rapid screening method for identifying cyanide in blood samples

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Background & Aims: Cyanide, a harmful substance to both humans and animals, has a molecular arrangement of one carbon atom and one hydrogen atom (CN). Currently, cyanide is being abused due to its hazardous characteristics, serving purposes such as suicide, criminal activities, and animal poisoning. However, some laboratories in some areas may have restrictions on verification. Therefore, the expediency and reliability of screening cyanide analysis are crucial. It is essential to utilize established protocols and enhance analytical methods. This study aims to evaluate the usability of a method for screening cyanide analysis in blood samples using a field water cyanide test kit, with the goal of reducing testing duration and providing a means to quantify cyanide levels through color stripe comparison. The data obtained from this study will serve as an important basis for future studies. This will lead to an increase in the potential for toxicological analysis.

Methods: Determination of appropriate equipment

The test was performed on a cyanide sample with a concentration of 250 ppb. The equipment used was compared among a 12 mL test tube, a 15 mL tube and a 50 mL erlenmeyer flask using 1500 µL of 1 M sulfuric acid and a total volume of 5 mL.

The determination of the amount of 1 M sulfuric acid and the appropriate total volume of the sample.

Tests were conducted on a 250 ppb cyanide sample in a 50 mL erlenmeyer flask. The volume of 1 M sulfuric acid was compared between 1500 and 3000 µL and the optimal total volume was compared between 5 and 10 mL.

Evaluation of the limit of detection (LOD).

Cyanide samples were prepared at various concentrations by preparing a 10 ppm of cyanide stock solution. Each concentration was repeated three times.

False positive test

This approach was adopted due to the potential occurrence of false positive results in the test. These substances included Cypermethrin, Alogliptin, Vildagliptin, Escitalopram, Febuxostat, and Verapamil, and were tested following the same procedure as cyanide testing. Samples were tested at concentrations of 250, 1000, and 2000 ppb in blood samples, with each test repeated three times in total.

Results & Discussion: Determination of appropriate equipment, amount of 1 M sulfuric acid and total sample volume for the method of testing for cyanide in blood samples with a MU test kit.

In this research, the selection of appropriate equipment revealed that the most suitable device was a 50 mL Erlenmeyer flask with dimensions of 3 cm in mouth diameter, 5 cm in base diameter, and 9 cm in height, which demonstrated the only positive results. In previous study, Humberto Estay et al. found that the transfer rate of HCN from the liquid state to the gas state occurs at the interface surface. The mass transfer rate depends on the diffusion coefficient of HCN, the dimensions and characteristics of the device. By determining the amount of 1 M sulfuric acid and the total volume of the sample by testing in a 50 mL Erlenmeyer flask, we determined that the most appropriate test would be the 1M of sulfuric acid, a volume of 3000 µL, with a total volume of 5 mL, for at least 10 minutes.

Evaluation of the limit of detection (LOD).
Dilute cyanide samples at various concentrations from a 10 ppm Cyanide stock solution. Repeat the dilution procedure three times for each concentration. Our result determined that the limit of detection of cyanide in blood samples is 75 ppb.

False positive test
False positive results were found in 6 samples: cypermethrin, alogliptin, vildagliptin, escitalopram, febuxostat and verapamil. The experiment was performed according to the same procedure, with a total of 3 repeats. False positive results were found that all negative results. This could be attributed to the presence of aromatic compounds containing nitrile in the test substances. The nitrile group is typically strong and tends to endure unchanged during metabolic reactions.

Conclusion: The study determined the most suitable equipment for testing, which included a 50 mL Erlenmeyer flask containing 1 M sulfuric acid, 3000 µL volume, and a total volume of 5 mL, with results observed after 10 minutes. Six concentrations of cyanide samples were tested, ranging from 75 to 2000 ppb, corresponding to 0.075 to 2 mg/L, respectively. These parameters were examined in the limit of detection (LOD) section, revealing an LOD value of 75 ppb. Additionally, the researcher investigated false positive results in all six samples—cypermethrin, alogliptin, vildagliptin, escitalopram, febuxostat, and verapamil—none of which yielded positive results. The preliminary setup can therefore be utilized for screening cyanide testing due to its simplicity, convenience, easy accessibility of equipment, lack of requirement for complicated tools, ability to preliminarily screen substances and compare quantities from color bands, low cost, and time-saving benefits compared to using an analyzer. However, additional parameters such as accuracy, precisionand the Metrix effect assessment, among others, require further investigation.

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Levels of endocrine disrupting chemicals in breast milk, infant's hair and feces

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Background & Aims: The rapid growth of the industry with the development of technology has increased exposure to synthetic chemicals. Among these compounds, endocrine disrupting chemicals (EDCs) have raised concern over the last years. EDCs cause adverse health effects by altering the functions of the endocrine system. These affect the growth and development of children in relation to the mother's nutrition, causing many adverse health problems. In this study, we aimed to detect EDCs in both breast milk and the hair and feces of breastfed children and to determine the level of exposure.

Methods: 103 mothers and their breastfed children were included in the study. The mean gestational age and weight of the infants was 38.6±1.3 weeks and 3.3 kg, 49.0% were male. The mean ages of the mothers were 29.5 ±5.5 years. We analyzed 153 compounds (147 pesticides, 5 polychlorinated biphenyls, and bis-2 ethyl hexzy phthalate) in breast milk obtained from mothers and infant's biological samples (feces and hair) both liquid chromatography-tandem mass spectrometry and gas chromatography-electrone capture detector. These compounds include 20 organophosphate insecticides, 7 pyrethroid and 14 carbamate insecticides, 14 organochlorine insecticides, 15 miscellaneous insecticides of undetermined classes, 39 fungicides and 38 herbicides; PCBs: 28, 52, 101, 118,153 and bis-2 ethyl hexzy phthalate, is the most common chemical among the phtalates. Organochlorine pesticides and polychlorinated biphenyls were only analyzed by gas chromatography-electron capture detector in breast milk and hair. The limits of detection (LOD) and quantition (LOQ) were calculated for pesticides and PCBs in hair, feces and breast milk.

Results & Discussion: The concentration ranges for pesticides and PCBs in breast milk, hair, and feces were 5-120 ng/mL, 20-500 pg/mg, and 10-1000pg/mg, respectively. Recoveries for EDCs were higher than 65%. Pesticides were detected in 39 of feces samples (48.7%), 17 of the infants hair (17.3%), 50 of the breast milk (50%). The highest rate of PCBs were found in breast milk and also PCB 28 and 52 were the most common among PCBs. Flutriafol, propiconazole, prothiofos, are the most frequently detected substances among pesticides. Bis-2 ethyl hexyl phthalate was detected in almost all samples.

Conclusion: Endocrine disrupting chemicals have been observed in breastfed infant's biological samples in association with chemicals detected in breast milk.

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Misuse of tramadol among drug addicts: A prospective study at CHU SETIF-Algeria

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Background & Aims: Tramadol is a centrally acting opioid analgesic with two mechanisms of action, including weak activation of the μ-opioid receptor by the drug, more potent activation by its main metabolite, and inhibition of serotonin and norepinephrine reuptake. Tramadol is considered an atypical opioid with less abuse potential compared to conventional opioid analgesics. However, some observational studies indicate that patients treated with tramadol have risks of chronic opioid use, similar or slightly higher, compared to those treated with other short-acting opioids. This study aims to determine the prevalence of misuse of oral tramadol products among subjects consulting at specialized addiction care center (CISA and psychiatric centers) in the Sétif province.

Methods: This prospective and analytical study, conducted over a five-month period from January to May 2023, examined urine samples from subjects consulting at intermediate addiction care centers in Sétif. A detailed information sheet was used to collect essential data. Participants were interviewed individually in the toxicology laboratory. Collected information included sociodemographic data, frequency of use, date of last intake, and combined use with alcohol, tobacco, or other drugs (Cannabis, Opioids, Benzodiazepines, Cocaine, and Barbiturates). Urine samples were collected under supervision in single-use polypropylene containers, a Samples were stored at +4°C for immediate analysis or frozen at -20°C for later analysis. Analyses included immediate screening using immunochemical techniques for tramadol and other drugs. Subsequent confirmations were performed using gas chromatography, employing SHIMADZU NEXIS GC-2030 equipment coupled with a GC/MS-QP2020NX mass spectrometer.

Results & Discussion: In our study, 71 subjects were included. Urinary analysis revealed 9 positive cases for Tramadol, accounting for 12.7%, and 22 subjects admitted to the misuse of this medication, resulting in a frequency of 31%. The average age was 23 years, ranging from 14 to 39 years. The age groups of 13 to 20 years and 21 to 30 years were the most represented, each accounting for 44.4% of the population. Males were predominant with a sex ratio of 10.08. The most represented category was single individuals at 91%. Unemployed subjects constituted 51.1% of the sample. In terms of control, 5 out of 71 requests were received, representing 7.04%, while 96.24% of the requests were for initial screening. Of the 22 subjects who admitted to misuse, 27.3% started more than five years ago, with 72% reporting regular consumption. Over 90% of cases combined tramadol with pregabalin, 22% with alcohol, and 90% with tobacco. Combinations of Tramadol with other psychoactive substances mainly included cannabis (36.4%), benzodiazepines (4.5%), cannabis and cocaine (4.5%), cannabis and benzodiazepines (22.7%), cannabis with amphetamines (4.5%), cannabis/cocaine/amphetamines (4.5%), and benzodiazepines with amphetamines (9.1%). No statistically significant correlation was established between tramadol use and certain studied variables such as sex, employment status, family situation, and the use of alcohol, tobacco, or other drugs (Fisher's exact test with $p>0.05$). The results of this study reveal several points of interest and concern regarding tramadol consumption habits in the studied population, while raising questions for future research. Firstly, the relatively young average age of participants (23 years) and the high prevalence of misuse among young adults (the age groups of 13 to 30 years represent the majority) compared to other studies, for example, the misuse prevalence in the prospective study by Scott A Reines et al, conducted in the USA between 2015-2017 found an average rate of 4%, highlight the need for targeted interventions for this population. The male predominance and high rate of singles suggest that specific social dynamics or stress factors in these groups might contribute to the use of this substance or drugs. The fact that the majority of subjects are unemployed potentially indicates a link between socio-economic challenges and substance use. This could be explored further in subsequent studies to understand whether consumption is a means of escaping difficult economic realities or if it contributes to the inability to obtain or retain employment. The predominant use of tramadol in combination with pregabalin, alcohol, and tobacco indicates a trend towards polydrug use, which can significantly increase health risks. The associations with illicit substances like cannabis, cocaine, and amphetamines underscore the need for integrated prevention strategies that address the consumption of multiple substances simultaneously. The lack of a statistically significant correlation between tramadol consumption and various demographic and social factors suggests that the use of this substance could be widespread across different subgroups of the population, highlighting the need for universal preventive approaches in addition to targeted interventions.

Conclusion: In conclusion, this study highlights significant issues related to Tramadol misuse in a young and predominantly male population. It underscores the need for prevention and intervention strategies that consider social, economic, and polydrug dynamics. To deepen the understanding of contributory factors and develop effective interventions, future research should explore the underlying causes of misuse of certain psychotropic drugs, including tramadol, and the mechanisms through which interventions can be most effective.

Development of AI-enhanced strip-type drug detection kits

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Background & Aims: The escalation of criminal activities related to drug use, particularly incidents where individuals are drugged through their beverages, underscores a pressing concern for public safety. A notable case in 2019 at a

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club in Gangnam, Korea, spotlighted this issue when drugs were clandestinely added to drinks, leading to addiction among women. This alarming trend has spurred the development of a novel solution: a portable, strip-type drug detection kit designed for beverage testing. To enhance the objective reading of the result, artificial intelligence(AI) technologies have been integrated. This AI-driven approach aims to refine the accuracy of the detection process.

Methods: A comprehensive analysis of various colorimetric reagents, including Dragendorff and Iodoplatinate, was conducted. Iodoplatinate emerged as the most suitable reagent for this purpose due to its sensitivity and specificity, and it was subsequently integrated into a strip-type drug detection kit.

The strip-type drug detection kit comprises several layers: a backing card, a nitrocellulose membrane that acts as the reactive surface, and an absorption pad, all contained within a 6mm by 80mm strip. A distinct window in the middle of the strip-type drug detection kit reveals the test results, indicating a positive or negative outcome based on the presence of target drugs. The selection of methamphetamine, ketamine, and cocaine as the focus of this study was informed by their prevalence and public health impact.

Confirm the strip-type drug detection kit's detection threshold, solutions of the three drugs were prepared in concentrations ranging from 0.01% to 0.5% in water. The change in the strip-type drug detection kit's coloration, indicative of drug presence, was assessed through RGB color metrics, a method that utilizes the primary colors of light (red, green, and blue) for color representation. To evaluate the strip-type drug detection kit's performance across various beverage matrix effect, 25 different types, including wine, whiskey, and grape juice, were spiked with a 0.5% concentration of each drug and tested.

The application of AI technology marked a significant advancement in interpreting the test results. Photographs of the strip-type drug detection kit was analyzed using a k-fold cross-validation method to train the AI, enhancing the accuracy and reliability of the detection process. It was compared against human judgment, with a panel of 32 police officers, comprising both crime scene investigators and general officers, evaluating 100 randomly selected test outcomes.

Results & Discussion: The development of a strip-type drug detection kit utilizing Iodoplatinate was proven to enable rapid detection of drugs in beverages, with vivid color changes observable within 30 seconds of application. With this kit, methamphetamine, ketamine, and cocaine were detected, with sensitivity levels reaching as low as 0.03% for methamphetamine and 0.02% for ketamine and cocaine.

The visual differentiation of these drugs is facilitated by distinct color changes on the test strip-type detection kit: methamphetamine produces a dark gray color at the bottom of the viewing window, ketamine results in a light gray, and cocaine is indicated by a gray color with a subtle purple color. The measurement of RGB values—a method employing the primary colors of light (red, green, and blue) to display color—revealed a linear relationship between the concentration levels of methamphetamine and ketamine and the corresponding color change. However, this linear correlation was not observed for cocaine.

In testing the strip-type drug detection kit across a variety of beverage types, the results demonstrated a high success rate, detecting all three drugs in 19 out of 25 tested beverages. False positives were identified in four beverages—orange juice, rice wine, beer, and yogurt—while soy milk and chocolate milk proved to be incompatible with the test. Out of 1419 tests conducted across these three drugs, 1196 returned positive results, and 223 were negative.

The incorporation of AI enhanced the interpretation. After training the AI with the data of positive and negative outcomes, it achieved a 98% accuracy rate when applied to the 1196 positive tests. This contrasted with the relatively lower accuracy of 74% observed among the 32 police officers who evaluated the same set of results.

Conclusion: The strip-type drug detection kit, utilizing iodoplatinate, represents a breakthrough in the rapid identification of amines in beverages, providing results in under 30 seconds. This strip-type drug detection kit has demonstrated the capability to detect methamphetamine at concentrations exceeding 0.03%, and both ketamine and cocaine at levels above 0.02%. Out of the 25 beverages tested, the kit proved effective for 19, showcasing its broad applicability.

In this study, it is significant that the results have been improved by applying AI, achieving an impressive 98% accuracy rate in distinguishing between positive and negative outcomes. This significantly surpasses the rates achieved through human assessment alone, highlighting the potential of AI in reading the results.

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Empowering bioanalytical methods: Development of a validation software using Python and SQL

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Background & Aims: Statistical analysis plays a crucial role in the validation of bioanalytical methods, enabling precise interpretation and extraction of valuable insights from complex data. Python, known for its simple syntax and readability, has become a popular choice among data scientists and biochemical analysts. Besides, SQL (Structured Query Language) plays a fundamental role in efficiently manipulating databases. The combination of Python and SQL programming languages offers a powerful and versatile approach to conducting the validation of an analytical method. The aim of this work is to develop a software to facilitate data analysis during the validation of analytical methods.

Methods: The Python and SQL programming languages will be used to create the software. The main objective is to create a user-friendly program that simplifies the validation process, requiring minimal or no training to operate. Data from a previously published paper from the first author was used to evaluate the applicability of the software. The validation criteria for the analytical method were based on the ANSI/ASB standard 036 guideline.

Results & Discussion: Microsoft SQL Server was used to create the software and, in order to evaluate if the software works, values from a paper published by the first author at the Journal of Analytical Toxicology (DOI: 10.1093/jat/bkaa138) was used, but in this case the parameters were evaluated using Excel®. The recovery, matrix effect and bias parameters were evaluated and are in accordance with the published paper. The next step is to add all the validation parameters in the software and compared to the results of the previously published method.

Conclusion: The use of Python could provide robust tools for data manipulation, statistical calculations and visualization. By combining Python for data manipulation and SQL for querying databases, we will be able to perform a rapid and accurate statistical analysis during the validation of quantitative methods. This software could contribute for significant advancements in the development and validation of bioanalytical methods.

Managing high-throughput demands in a forensic toxicology laboratory – the application of the Toyota Production System for reducing turnaround times for random roadside drug testing in Victoria, Australia

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Background & Aims: Since 2004, Victorian drivers have been subject to random roadside drug testing in oral fluid for Methylamphetamine, 3, 4-Methylenedioxy-N-Methamphetamine (MDMA) and Delta-9-Tetrahydrocannabinol (Δ9-THC). In 2008, Victoria Police performed 25,006 roadside drug tests with 438 (1.8%) drivers testing positive to drugs; this contrasts with 2022 where roadside drug testing increased to 167,648 with 10,628 (6.3%) drivers testing positive. All samples collected at the roadside following a positive test are subject to confirmatory testing by the toxicology laboratory at the Victorian Institute of Forensic Medicine (VIFM). The management of over 10,000 confirmatory analyses to a forensic standard has considerable challenges to ensure traceability, integrity and defensibility. The aim was to reduce the turnaround times (the time between sample receipt and issuing of the report) for these cases in accordance with stakeholder needs, consultants from the Toyota Production System Support Centre (TSSC) were engaged to review and improve oral fluid processes by applying the Toyota Production System (TPS) principles.

Methods: Oral fluid specimens collected by Victoria Police from drivers testing positive at the roadside were transported to the VIFM for confirmatory analysis. All specimens were receipted and entered into the internal case management system before undergoing liquid-liquid extraction followed by analysis using liquid chromatography tandem mass spectrometry. Positive detections were reported above the cut-off concentrations listed in the Australian standard AS4760:2006. This existing process took an average of 9 days to complete due to fluctuating receipt of samples, delays in numerous parts of the process and other competing laboratory tasks.

The TPS is an integrated socio-technical system, that comprises of three parts, Management, Technical and Philosophy. The TPS is based on the philosophy of achieving the complete elimination of waste in pursuit of the most effective methods. The main objectives are to reduce stress (muri) and inconsistency (mura) and to eliminate waste (muda). Problems tackled included examination of laboratory resources (equipment and staff), stagnation and bottleneck actions such as the inconsistent number of oral fluid samples conveyed per day hence under-utilising the maximum number of extractions routinely performed.

Results & Discussion: The number of oral fluid samples receipted into the laboratory pre and post the application of the TPS tools remained consistent at approximately 900 samples per month. Initially a scheduling system was introduced to level the work volume, create continuous flow and assist in the identification of laboratory problems. The review identified all the steps required for a successful completion of an oral fluid analysis by prioritising and scheduling the workload per person per day. Secondly, visual boards and cards (kanbans) were developed which identified stagnation points for assays and problems with equipment. Thirdly the maximum number of oral fluid specimens extracted per day was optimised by topping up analytical assays with additional receipted specimens on the day. This "top up" approach further levelled the sample throughput and ensured maximum efficiencies within assays by reduction of sample stagnation. Overall, the project took 18 months to complete with guidelines developed and adopted by both toxicologists and management to ensure an improved (kaizen) and successful streamlined operation. The laboratory's average turnaround times for oral fluid reports before the introduction of TPS tools was 9 – 10 days with 90% of reports completed within 12 days. After the application of the TPS tools, average turnaround times decreased to 4 days with 90% of reports completed within 5 - 6 days. While the workload and cost of testing remained similar, samples were analysed more effectively with increased capacity for staff to identify and plan their day more productively.

Conclusion: With the assistance of the TSSC the laboratory implemented and endorsed the improved oral fluid confirmation service which resulted in an average decrease in turnaround times of 58%. This process enhanced stakeholder satisfaction and laboratory workflows without additional resources. Most importantly, the TSSC provided tools for toxicologists to reduce the 3M (muri, mura and muda; TPS concepts), reduce the unevenness of the entire analytical process (heijunka) and reduce overall turnaround times. This project highlights how modern forensic toxicology laboratories must better utilise available resources to keep up with increasing demands from all stakeholders (e.g. coroners, police).

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Did you ever hear of Esterom®?

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Background & Aims: In the presented case, a car driver explained his positive benzoylecgonine level in a DUI case by taking a medication named Esterom®. A dental practice confirmed in writing that dental treatment had not been possible due to a jaw clamp. After prescribing the drug Esterom, allegedly the symptoms had alleviated and treatment had become possible. A close temporal connection between driving and the application could not be established because the medical opinion was undated.

Methods: We applied our routine method (immunoassays and GC-MS, liquid-liquid extraction, and derivatization) to the blood specimen.

Results & Discussion: Toxicological analyses revealed levels of THC of 4.8 µg/L, 11-OH-THC of 2.5 µg/L and THC-carboxylic acid of 24 µg/L as well as 110 µg/L of benzoylecgonine.

Esterom® solution, formerly an investigational pharmaceutical drug, is derived from the esterification of benzoylmethylecgonine (cocaine) with 1,2-propanediol. The resulting solution contains a mixture of a number of components except for cocaine. Esterom® solution was intended to be a topical analgesic to relieve pain and increase range of motion in patients suffering from acute inflammation of the shoulder or back. All constituents of Esterom® are known. Among the products there is also benzoylecgonine, while all cocaine is removed. Applied topically to rats, ecgonidine, methylecgonidine, benzoylecgonine, and hydroxypropyl ecgonidine were not detected. Esterom® or a similar drug is not approved as a medication in Germany.

Conclusion: We wondered how a drug unknown to our university dental clinic and at least one practitioner, which was not approved by the FDA in the USA due to a lack of proof of efficacy, whereupon the company Entropin was facing securities fraud class action by investors in 2003 settled in 2004 by paying $ 4,5 million and which was dissolved in 2007, was allegedly used in a German dental practice in 2021 with an indication which it was never developed or tested for. It never obtained an approval by the European Medicines Agency EMA. We have advised the district's driving licence office accordingly, but have not been informed about the progress of the procedure.

DIV P-32

Will oral-ingested cannabinoids react with acid in the stomach when combined with alcohol?

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Background & Aims: In 2016, J. Merrick et al. reported that orally ingested CBD may be converted to THC in the human stomach. However, some researchers questioned Merrick's result, and further research revealed that some additives in the simulated gastric fluid in Merrick's research promoted conversion reactions. Moreover, the conversion reaction has not been confirmed through in vivo digestion experiments of CBD. For these reasons, it has been widely accepted that CBD would not be converted to THC in the human stomach. However, there is no research examining whether this conversion reaction can occur when CBD is consumed with food and drink. Additionally, there is limited published research on the behavior of other cannabinoids in the stomach. Therefore, this study aimed to elucidate whether CBD, Δ9-THC, and Δ10-THC, known to be vulnerable to acid will react with stomach acid through an in vitro digestion experiment when combined with alcohol.

Methods: Simulated gastric fluid was prepared according to the in vitro digestion experiment in a flask conducted by Kozu. Each cannabinoid was then added to the solution assuming that the amount of ingestion was from 25 mg to 100 mg. To investigate the effect of the combined use of alcohol, ethanol was added to the solution so that the ethanol concentration became between 0 % and 20 % in simulated gastric fluid, and digestion experiments were conducted for each ethanol concentration in a constant temperature bath. After digestion experiments, each solution was neutralized with a base and was extracted with ethyl acetate. The conversion rate of each cannabinoid was evaluated by using GC-FID.

Conditions of digestion experiments
Simulated gastric fluid 2 mL (pH 1.3 (HCl), NaCl 18 mg), Each cannabinoid 0.1 mg to 0.4 mg (assuming ingestion of 25 mg to 100 mg); Temperature: 37 °C; The speed of shaking: 115 strokes/min; Shaking time: 180 min

Conditions of GC-FID
Equipment: Agilent 6890; Column: HP 5(Agilent Technologies; 30 m×0.32 mm I.D., 0.25 mm)

Results & Discussion: In the digestion experiment assuming 25 mg ingestion, no conversion reaction of Δ9-THC was observed regardless of ethanol concentration. On the other hand, it was confirmed that CBD and Δ10-THC were partially converted into Δ9-THC and Δ6a(10a)-THC. Furthermore, a positive correlation was obtained between ethanol concentration and conversion rate, and about 3 % of CBD and 9 % of Δ10-THC were converted at 20% ethanol concentration. Δ10-THC has the highest conversion rate among the three, which is why the conversion reaction proceeds via a more stable benzyl cation intermediate.

In the digestion experiment assuming 100 mg ingestion, the conversion rate significantly decreased, and only 1 % of CBD was converted into Δ9-THC even at 20 % ethanol concentration. We consider that this significant decrease is due to the low solubility of CBD in simulated gastric fluid. This low solubility means that only a small fraction of added CBD could be involved in the reaction, as a result, the conversion reaction proceeds only very slightly regardless of the ingested amount.

Conclusion: 1. CBD and Δ10-THC can be partially converted into Δ9-THC and Δ6a(10a)-THC in the human stomach when used in combination with alcohol.

2. Due to the low solubility of cannabinoids in gastric fluid, the conversion reaction of cannabinoids in the stomach proceeds only very slightly regardless of the ingested amount.

Poster gallery - PM F-P-1 to P-48

10:00 - 10:30 Friday, 6th September, 2024

Redistribution of amlodipine in postmortem samples

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PM F-P-01

Background & Aims: This study evaluates the postmortem redistribution of amlodipine in an 82-year-old hypertensive woman who died en route to her home, focusing on the challenge of correlating postmortem drug concentrations with antemortem therapeutic levels. It emphasizes the need for advanced analytical methods to accurately distinguish between therapeutic use and potential overdose in deceased individuals.

Methods: Postmortem samples, including heart blood, femoral venous blood, liver tissue, and stomach contents, were collected at various intervals. These samples were analyzed using state-of-the-art liquid chromatography-mass spectrometry (LC-MS), focusing on protein precipitation, analytical procedures, and quantification methods. The sensitivity of the analysis was enhanced by using a Waters BEH C18 column, acidic acetonitrile and ammonium acetate as mobile phases, and an electrospray ionization source in multi-reactive ion monitoring mode. The calibration curve spanned 0.5 to 200 ng/mL, with a detection limit set at 0.5 ng/mL.Postmortem samples, inclu-

ding heart blood, femoral venous blood, liver tissue, and stomach contents, were collected at various intervals. These samples were analyzed using state-of-the-art liquid chromatography-mass spectrometry (LC-MS), focusing on protein precipitation, analytical procedures, and quantification methods. The sensitivity of the analysis was enhanced by using a Waters BEH C18 column, acidic acetonitrile and ammonium acetate as mobile phases, and an electrospray ionization source in multi-reactive ion monitoring mode. The calibration curve spanned 0.5 to 200 ng/mL, with a detection limit set at 0.5 ng/mL.

Results & Discussion: The analysis revealed amlodipine concentrations significantly above therapeutic peaks (14 ng/mL), with initial heart blood levels at 27.5 ng/mL to 209.7ng/mL after two months, indicating extensive postmortem redistribution. The concentration of amlodipine in venous blood, liver tissue, and stomach contents extracted after two months was 209.7ng/mL,117.2ng/mL, 2698ng/g, 801ng/mL, respectively. Concentrations in heart and femoral blood showed progressive increases, while liver and stomach contents remained consistent with daily therapeutic doses. This pattern underscores the importance of multi-matrix sampling in forensic toxicology. Despite suspicions of overdose, further investigations confirmed the absence of excessive amlodipine intake prior to death.

Conclusion: The advanced LC-MS techniques applied in this study highlight the complexity of interpreting post-mortem drug concentrations, especially for drugs with significant redistribution properties like amlodipine. These findings caution against direct comparisons of postmortem drug levels with antemortem therapeutic concentrations and advocate for the use of sophisticated analytical tools and comprehensive sample analysis to improve accuracy in forensic interpretations. Ongoing research and broader biological sampling are recommended to refine forensic assessments in drug-related fatalities.The advanced LC-MS techniques applied in this study highlight the complexity of interpreting postmortem drug concentrations, especially for drugs with significant redistribution properties like amlodipine. These findings caution against direct comparisons of postmortem drug levels with antemortem therapeutic concentrations and advocate for the use of sophisticated analytical tools and comprehensive sample analysis to improve accuracy in forensic interpretations. Ongoing research and broader biological sampling are recommended to refine forensic assessments in drug-related fatalities.The advanced LC-MS techniques applied in this study highlight the complexity of interpreting postmortem drug concentrations, especially for drugs with significant redistribution properties like amlodipine. These findings caution against direct comparisons of postmortem drug levels with antemortem therapeutic concentrations and advocate for the use of sophisticated analytical tools and comprehensive sample analysis to improve accuracy in forensic interpretations. Ongoing research and broader biological sampling are recommended to refine forensic assessments in drug-related fatalities.

PM F-P-02

Gastric content analysis as a forensic tool in delayed pesticide toxicity deaths: A case series from South Africa

Bronwen Davies
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Background & Aims: In South Africa, the illegal sale of toxic agricultural pesticides for domestic use ('street pesticides') poses significant public health challenges. Diagnosing pesticide poisoning post-mortem, and further identifying the specific pesticide involved, may be challenging in cases where the deceased survived under medical care for extended periods, and hospitals have discarded antemortem specimens. This study emphasizes the important role of gastric content analysis in post-mortem toxicology, especially for cases of delayed pesticide-related deaths, offering valuable insights that can guide forensic investigations and inform public health strategies in resource-limited settings.

Methods: A retrospective, descriptive review was performed on all post-mortem cases in Western Cape province submitted to the Forensic Toxicology Unit for pesticide screening from January 1st, 2023, to February 29th, 2024. Cases were included for further review if individuals survived in the hospital for any period (excluding individuals who were dead on arrival at the hospital). All cases underwent a pesticide qualitative screen using a Waters XevoTM TQD UPLC-MS/MS targeted panel for 24 organophosphate and carbamate pesticides, the results of which were included in this review.

Results & Discussion: A total of 73 cases were submitted to the Forensic Toxicology Unit, 22 (30.1%) were hospitalised, with 14 exhibiting cholinergic toxidrome symptoms (such as salivation, lacrimation, miosis, bronchorrhea, fasciculations, sweating, abdominal pain, and seizures) consistent with organophosphate and/or carbamate toxicity. Only post-mortem specimens were submitted in the 14 cases: femoral blood and gastric contents (n=7), femoral blood only (n=5), and gastric content only (n=2). Gastric content analysis, performed in a total of nine cases, revealed the presence of the organophosphate terbufos and/or terbufos sulfoxide in all instances, even when these analytes were not detected in blood. Where only blood was submitted, only 2 (40%) cases were positive (again for terbufos and/or its metabolite). This case series highlights the longer detection window of this pesticide in gastric contents compared to blood, with analytes identifiable up to eight days post-ingestion, despite hospitalization.

Conclusion: This review highlights the critical role of gastric content analysis in forensic toxicology, especially in cases of pesticide poisoning with extended hospital stays before death. Qualitative analysis of gastric contents not only confirmed the presence of the toxic analyte but also supported the clinical diagnosis. The identification of the pesticide involved is important in the South African context where the illegal purchase and domestic use of highly hazardous pesticides, such as terbufos (classified as Class 1A Extremely Hazardous), is prevalent in poorer communities. This availability increases the risk of accidental ingestion among young children and use in suicidal attempts by teenagers and adults. It is advised that forensic pathologists collect post-mortem gastric content in cases of pesticide-related fatalities, regardless of the duration spent in the hospital. This approach is vital for post-mortem diagnoses and carries significant implications for public health strategies and preventive measures against pesticide poisoning in South Africa and similar settings.

Death by cannabis

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Background & Aims: In agreement with literature data, the risk of death due to direct cannabis intoxication appears negligible. However, there are some cases where its use is linked to the possibility of fatal events due to cardiac complications or physical injuries procured in an altered conscious state, but the most relevant cases involve adults with preexisting heart disease, rather than children.

The authors present here a fatal case of a toddler that was taken by his parents to the nearest hospital due to the onset of alterations in walking, balance, and consciousness. On arrival, health care providers learned from the father that his son had allegedly ingested a substance having the color of cork and the consistency of soil. According to his account, such ingestion occurred that morning while he was playing at the playground located near their home. Despite all the therapies implemented by the medical staff, his condition was progressively worsening with onset of bradypnea and then dyspnea requiring intubation. Two hs later death was noted. During the search carried out by judicial police officers at the family's home, a modest amount of hashish was found, of which the father appeared to be a user.

Methods: At the hospital, a sample of blood was collected for clinical investigation. An autopsy was ordered by the judicial authority, The autopsy specimens were subjected to a post-mortem toxicological screening battery. Peripheral blood sampled at the emergency room (peripheral blood) was also included in this analysis. Narcotic and psychotropic substances were screened in cadaveric urine by immunoassay. Xenobiotic substances including drugs of abuse, pharmaceutical drugs and poisons of vegetal origins were searched in living blood, cadaveric blood, urine, and bile by ultra-high pressure liquid chromatography multiple mass spectrometry (UPLC-MS/MS) on a Xevo TQS Waters. THC, 11-OH THC and THC-COOH were quantified in fluids and tissues by liquid/liquid extraction, derivaztization and gas chromatography-mass spectrometry (GC-MS) on an Agilent 5973 instrument

A genetic analysis was also conducted on the heart muscle of the deceased for the research of mutations eventually related to the development of sudden arrhythmic events.

Results & Discussion: Histological investigations and genetic analyses were inconclusive.

Autopsy evaluation revealed a nonspecific picture of multi-organ congestion involving the encephalon, lung, liver, and kidney. Toxicological analysis detected cannabinoids in blood drawn at the emergency room. Cannabinoids were also identified and quantified in postmortem central blood, peripheral blood, urine, bile, brain, lung, and liver samples.

THC was found at 14 ng/mL, 3.6 ng/mL, 4.6 ng/mL, and 19 ng/mL in peripheral blood, central blood, urine, and brain respectively.

The active metabolite 11-OH-THC was found at 11 ng/mL, 3.8 ng/mL, 60.8 ng/mL, 9.8 ng/mL in peripheral blood, central blood, urine, bile and liver respectively.

The carboy metabolite THC-COOH was present at 106 ng/mL, 40 ng/ml, 51 ng/mL, 248 mg/mL, 1.2 ng/mL, 81 ng/mL 40 ng/mL in peripheral blood, central blood, urine, bile, brain, liver and lung respectively.

Hair analysis (3 cm long hair) showed the presence of tetrahydrocannabinol at 910 pg/mg, cannabidiol, cannabinol, methadone and metabolite, cocaine and metabolites, morphine and 6-monoacetylmorphine, revealing previous exposures to drugs of abuse, possibly corresponding to his parents habits.

Conclusion: Acute cannabis intoxication over a framework of chronic exposure to numerous drugs was considered responsible for the death of the child. The absence of pre-existing conditions alone capable of causing his death, the circumstantial data, the absence of other possible causes, and the levels of the substance and its metabolites detected in the victim's fluids and tissues support this statement.

In the frame of legalization of cannabis, an increasing number of cannabis intoxication cases are expected worldwide. Data on younger individuals are still scarce, but from the presented case no doubt arises that cannabis can kill.

PM F-P-04

Anabolic steroids: "Deadly poisons"

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Background & Aims: Demonstrate the responsibility of anabolic steroids in the death of a bodyguard found dead in his bed with no signs of trauma and in the presence of several boxes of anabolic steroids (Mesterolone, Oxandrolone).

The autopsy revealed signs of asphyxia with no sign of violence. Given the occupation of the subject, the pathologist suggested a possible toxic death.

Methods: Screening for narcotics, new synthetic products (NSP), and drugs was carried out using LC-MS-MS on a XEVO TQS-micro (Waters) coupled with tandem mass spectrometry.

For anabolic steroids, extraction of analytes from the biological matrices was achieved using liquid-liquid extraction in a mixture of organic solvents (ether/dichloromethane/hexane/isoamyl alcohol) at pH 8.4. After decontamination using dichloromethane, black hair (4 cm long) was cut up finely and incubated in 1 mL of methanol and 1 ng of testosterone-d3 (EI), sonicated for 90 minutes, and then centrifuged (15 min., 3000 rpm). Before extraction, urine was hydrolysed. Hair was first decontaminated, then pulverized. All methods are validated and published.

Results & Discussion: During the autopsy, samples such as femoral blood, urine, hair, cardiac blood were collected and sent to the laboratory.

No alcohol, narcotics, psychotropic drugs, or NSP were detected in the blood and urine of the deceased.

Screening for anabolic steroids in the blood found traces (< 1 ng/ml) of androstenedione, boldenone, nandrolone, and oxandrolone. DHEA (9 ng/ml), DHT (5 ng/ml), and testosterone (5.4 ng/ml) were identified at physiological concentrations. In the urine, boldenone (0.2 ng/ml), oxandrolone (50 ng/ml), and nandrolone (2 ng/ml) and its metabolites, norandrosterone (51 ng/ml) and 19-noretiocholanolone (23 ng/ml), were detected. A total of 27 ng/ml of testosterone and 1 ng/ml of epitestosterone were detected. This T/E ratio of 27, which is higher than 4, is consistent with an exogenous source of testosterone.

The analysis of the hair revealed the presence of 2 200 pg/mg of androstenedione, 143 pg/mg of boldenone, 2100 pg/mg of DHEA, and 1700 pg/mg of testosterone.

This hair test demonstrates high and repeated consumption of testosterone, DHEA, androstendione and boldenone, and therefore, a chronic intoxication can be considered.

Conclusion: The analyses have established the regular and massive abuse of various anabolic steroids. These molecules are likely to cause cardiac or hepatic disease with potentially fatal consequences. In the absence of histological tests, the toxicological assessment seems essential establishing in this case a toxic death due to by steroid abuse.

PM F-P-05

But did they drink it?

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Background & Aims: Tetrahydrozoline (THZ) is a peripheral alpha-1 agonist used therapeutically as an eyedrop to reduce redness of the conjunctiva or as a local nasal decongestant. It is structurally similar to drugs such as clonidine and tizanidine. When ingested, it has central alpha-2 agonist effects as these drugs such as lethargy, bradycardia, hypotension, and respiratory depression. Cases of oral ingestion, primarily in children, have been reported in the literature, and there have been multiple cases of homicide linked to surreptitious oral administration. The aims of this study were: 1) Identify THZ positive blood cases from a large database of postmortem casework and compare the reported results to the existing literature; 2) determine vitreous humor (VH) concentrations of THZ in cases where it was quantified in blood; and 3) examine if the blood:VH ratio can be used to differentiate between ocular and oral exposure.

Methods: Data were extracted from NMS Labs' (Horsham, PA, USA) Laboratory Information Management System for all cases received from January 1, 2016 to February 29, 2024. Postmortem cases with a reported blood THZ concentration were identified and availability of VH for testing was determined. Permission was obtained from clients when necessary to test the VH samples. Summary statistics for blood THZ and VH results were calculated including the B:VH ratios. Case histories were reviewed to identify cases with known or suspected ingestion of THZ.

THZ quantification in blood is a fully validated method. Samples undergo liquid-liquid extraction and are quantified via liquid chromatography-tandem mass spectrometry. The analytical measurement range is 0.05-10 ng/mL. Samples with concentrations exceeding the highest calibrator are diluted with blank calibrator matrix and repeated. VH is not a validated matrix for the assay. For this study, quantification was achieved using three levels of standard addition when specimen volume permitted.

Results & Discussion: All samples with sufficient volume for standard addition were quantified against the serum calibration curve and by standard addition. The average percent difference between the standard addition quantification and the calibrator curve quantification was -9.3%. Based on the consistency between the two results, cases with insufficient volume for standard addition were quantified using the serum calibration curve.

The range, mean, median and interquartile range (IQR) for THZ in blood (N=133) were 0.19-2800, 45, 1.7 and 5.4 ng/mL, respectively. VH results (n=12) were 0.64-87, 23, 12 and 33 ng/mL for range, mean, median and IQR, respectively. B:VH ratios ranged from 0.040-7.6.

Three cases involving known or suspected THZ ingestion were identified. The B:VH ratio in these cases were 3.2, 5.2 and 7.6. Cause of death (COD) was determined to be acute toxicity from diphenhydramine and THZ in two of these cases, with suicide as manner of death. Two additional cases had ratios >1.0. COD for these cases were blunt force trauma (unrestrained driver involved in a car crash) and gunshot wound to the head (suicide). Half of the cases where VH was available had B:VH ratios <0.2, and these cases had no suspicion of ingestion.

Conclusion: Based on these limited data the B:VH ratio may be useful in differentiating between oral and ocular exposure to THZ. A more comprehensive study is necessary with the aim of including more samples with blood concentrations between 1.0 – 20 ng/mL. In addition, stability studies in VH will be performed.

Applying toxicological findings to unravel real cause of death for fatalities initially perceived as accidental – a case study

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PM P-06

Background & Aims: Understanding the role of forensic toxicology in completely incinerated bodies is essential for forensic practitioners, medical professionals and legal authorities. The investigation into the role of forensic toxicology may unravel details that might have contributed to the cause of death.

Postmortem samples from the extensively incinerated corpse of a 33 year old female were received along with a history of sudden residential fire. According to other five survivors residing in the same apartment, while they were waiting for breakfast with one of them playing with a cigarette lighter, there was an abrupt house-fire with intense smoke. The deceased female could not be evacuated and was found lying-dead on her bed. Her postmortem revealed that the cause of death was 100% burning due to flames. Blood, liver and stomach with contents were received in toxicology department. When investigating burned bodies, analysis of sedatives and carbon monoxide (CO) marks the initial phase of toxicological testing. Due to inconclusive findings in initial phase, an in-depth drugs screening was conducted.

The aim of this study is to shed light on the intricate interplay between toxicological analysis and postmortem findings, accentuating the pivotal role of forensic toxicology in unraveling the mysteries surrounding undetermined deaths.

Methods: Blood was screened for sedatives and carbon monoxide using immunoassay and colorimetric technique, respectively. Further, blood, liver and stomach contents were extracted using QuEChERS extraction with added clean-up step using Agilent EMR (C18) powder. Extracted samples were run on GC/MS in scan mode using DB-5MS (15 m × 250 µm × 0.25 µm). To perform confirmation and quantitation of cocaine and ecgonine methyl ester, liver and stomach contents were extracted using solid phase extraction (mixed mode: cation exchange/C18 sorbent) and derivatized with BSTFA. Extracted samples were run on GC/MS in SIM mode using DB-5MS (30 m × 250 µm × 0.25 µm).

Results & Discussion: Immunoassay was negative for sedatives. Unexpectedly, CO testing also yielded negative results. However, cocaine and cocaethylene were detected in blood, liver and stomach contents during initial drugs screening. Since the blood was already consumed during initial screening, therefore, quantitation test for cocaine was only performed on liver and stomach contents. 131 µg/Kg cocaine and 287 µg/Kg ecgonine methyl ester were found in liver; whereas, 1182 µg/L cocaine and 499 µg/L ecgonine methyl ester in stomach contents.

Under the circumstances when a body has undergone complete burning and there is no detection of CO, it implies that the individual likely did not inhale CO during the incident; which can be suggestive of individual's demise before the incident.

Cocaine has a half-life of 1-3 hours and the time frame for detection of cocaine in blood is relatively short. Generally, presence of high amounts of intact cocaine in stomach contents and any measurable amount of cocaine in blood and liver indicates recent exposure. Additionally, presence of cocaethylene highlights concurrent use of cocaine with alcohol and in this combination, the primary metabolism of cocaine to inactive benzylecgonine and ecgonine methyl ester is altered to produce predominantly cocaethylene (this transesterification of cocaine to cocaethylene is about 3.5 times faster than hydrolysis to benzylecgonine). The data suggests that while cocaethylene is cardiotoxic itself, co-administration of alcohol with cocaine leads to greater systemic absorption of cocaine culminating at exacerbated cardiotoxicity.

Consequently, provided there are no significant anatomical injuries evident in the postmortem findings, simultaneous identification of cocaine and cocaethylene in blood, liver and stomach contents, coupled with the absence of CO in extensively incinerated body infers that the cause of death is likely attributable to the toxic effects of cocaine and cocaethylene.

Conclusion: Forensic toxicology serves as a cornerstone in uncovering the true cause of death, particularly where circumstances are initially framed as accidental or natural. This study emphasizes the importance of interpreting toxicology findings for legal investigations; taking into account elements such as, confirming specific drug concentrations delineating timeline of substance exposure, the synergistic effects of cocaine with alcohol and their contribution to individual's death. Hence, the unforeseen toxicological findings in this case altered the entire initial narrative and thus, shifted the investigative landscape.

Also, findings of this study propose the incorporation of systemic toxicological analysis in fire-related deaths where carbon monoxide remains undetectable, to precisely establish exact cause of death.

PM P–07

Acetone and isopropanol analysis in forensic investigation

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Background & Aims: Ketoacidosis is a frequent complication of Diabetes Mellitus (DM), which can be fatal, leading to potential medicolegal implications. In the case of DM, the most severe metabolic issues include diabetic ketoacidosis (DKA), hyperosmolar coma, and hypoglycemia. A metabolic disorder similar to DKA but with a different cause is alcoholic ketoacidosis (AKA), which can be caused by prolonged exposure to alcohol.

Ketoacidosis is a potentially harmful condition caused by the uncontrolled production of ketone bodies (indicative of metabolic problems). In addition to DM, the most common causes of ketoacidosis stem from food deprivation and hypothermia. The three ketone bodies are acetoacetate, beta-hydroxybutyrate, and acetone, produced when the body's energy source shifts from glucose utilization to fatty acid breakdown due to insulin deficiency.

Acetone is formed through the spontaneous decarboxylation of acetoacetate and can further be converted to isopropanol through the enzyme alcohol dehydrogenase. In turn, isopropanol can also be metabolized to acetone.

Since the simultaneous detection of acetone and isopropanol in biological samples may indicate exposure to the latter volatile, several authors have suggested and demonstrated that the conversion between these two substances explains their simultaneous presence in post-mortem biological samples, especially in cases of diabetics, deaths due to hypothermia, or alcohol abuse-related deaths.

It is important to note that after death, various biochemical changes occur, and the metabolism of bacteria induces glycolysis and proteolysis processes during the putrefaction phase. Both acetone and isopropanol can be produced post-mortem by microorganisms, due to putrefaction. Thus, it is imperative to ascertain the specific scenarios (e.g., diabetic ketoacidosis, alcoholic ketoacidosis or due to factors like putrefaction), where quantification of these substances is necessary, given their frequent detection in routine ethanol analysis.

The purpose of this work was to deepen the study of these two substances in forensic contexts, especially when analyzing blood samples, to clarify the significance of confirming and quantifying these volatiles in routine procedures of a Forensic Toxicology Laboratory. For this the authors developed an analytical method of gas chromatography coupled with flame ionization detector and headspace injector (HS-GC/FID), incorporating 2 internal standards. After validation, the method was applied to all cases in which there was a suspicion of the presence of these volatile substances in analyses of alcohol screening.

Methods: For the analysis of the two substances in question, the methodology was developed using an Agilent 6890 GC with a flame ionization detector and coupled to an Agilent G1888 headspace injector of fixed volume of 1 mL (HS-GC/FID). This system is equipped with two analytical columns of different polarity (DB-ALC2: 30 m x 0.320 mm x 1.2 µm and DB-ALC1: 30 m x 0.320 mm x 1.8 µm) to unequivocally guarantee the presence of the mentioned volatile substances. Before conducting gas chromatography analysis, the samples, including the calibrators, were diluted 1:10. This involved diluting 100 µL of blood with 1 mL of an aqueous solution containing n-propanol (100 mg/L) and ethyl acetate (100 mg/L), which served as the internal standards.

The study will be conducted employing the utilization of two internal standards: n-propanol, widely employed as an internal standard in scientific literature and in the laboratory routine of the Service, and ethyl acetate, which, although it may suggest the presence of bacteria responsible for possible ethanol formation after death, it does not represent the prevailing volatile compound nor a robust indicator in post-mortem ethanol production scenarios. Furthermore, it is a substance that does not interfere with the ones to be studied. Thus, this choice becomes relevant to drive innovation in the work and to understand the aspects that will be addressed.

After validation, the method was applied to all cases in which there was a suspicion of the presence of volatile substances at the forensic toxicology service from the INMLCF, during two years.

Results & Discussion: The described method has been fully validated according to the guiding principles of the American Academy of Forensic Sciences Academy Standards Board (AAFS ASB).

The results demonstrate the method was selective and linear in concentration range of 50-4000 mg/L, with correlation coefficients exceeding 0.99. Detection limits ranged from 3 mg/L to 9 mg/L, and quantification limits ranged from 10 mg/L to 26 mg/L. No significant interfering compounds or carryover were observed for these two substances. Precision, both inter and intraday, met the standard criteria typically accepted during bioanalytical method validation. Evaluation of this parameter through the coefficient of variation, CV (%), resulted in values below 10% for the substances under analysis, ranging from 0.8 to 4.4%. Coefficients of variation, CV (%), were below the 20% criterion.

The authors analyzed all cases where there was a suspicion of the presence of the volatile substances in routine alcohol analyses. The method was applied to real cases and the determination of acetone and isopropanol was made in 110 cases of postmortem samples obtained from autopsies performed in the National Institute of Legal Medicine and Forensic Sciences of Portugal.

Conclusion: The authors hope with this study to demonstrate the importance of analyzing these substances for different situations (e.g., diabetic ketoacidosis, alcoholic ketoacidosis or due to factors like putrefaction).

Refining knowledge on propranolol overdosing through an autopsy case series

PM P–08

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Background & Aims: Propranolol is a highly lipophilic, non-selective beta-receptor antagonist, very effective and widely used for e.g. treatment of hypertension, cardiac failure and cardiac angina and for secondary prevention of myocardial infarction in adult patients. Besides for cardiovascular diseases, propranolol is used (off-label) for several other indications, such as prophylaxis of migraine or physical symptoms (palpitations, tremor) in anxiety disorders. When not used as prescribed, there is a high risk of potentially fatal poisoning due to its powerful cardiac effects and swift onset of action.

Recent epidemiological studies reported differing results on the share of propranolol intoxications among beta-blocker overdoses. Despite the availability of some published studies and case reports including clinical data, records of post-mortem concentrations and autopsy findings from propranolol overdosing remain relatively scarce. This evaluation is further complicated by the frequent occurrence of multi-drug intoxications involving propranolol, as opposed to single overdose. We describe four independent post-mortem cases of mixed drug intoxication, all including propranolol overdosing.

Methods: In each case, a forensic autopsy including histopathology and toxicological analysis was performed. Propranolol was measured in post-mortem whole blood collected in NaF tubes from the femoral vein, as well as in urine and stomach content samples, using a validated analytical method based on liquid-liquid extraction and liquid chromatography coupled to tandem mass spectrometry. The presence and concentrations of other medication and drugs of abuse were also determined using validated analytical methods based on chromatography and mass spectrometry.

Results & Discussion: All post-mortem cases occurred in 2022 and included three men and one woman, age ranging from 21 to 62. All persons were found deceased and cardiopulmonary resuscitation was not attempted. In three cases, several (partially) intact tablets containing propranolol were found in the esophagus, stomach contents and/or the duodenum. Furthermore, autopsy findings were non-specific. Pulmonary edema, present in all cases, likely indicated a protracted agonal phase. Toxicity from beta-blocker exposure typically manifests within 2 h of ingestion, when peak plasma concentrations are reached. However, the initial critical signs of overdose may emerge as early as 20 minutes post-ingestion, with the majority occurring within the first 2 to 4 h. Propranolol overdose can induce sinus bradycardia, hypotension and cardiac arrhythmias. This can lead to heart failure, cardiogenic shock or myocardial infarction. Besides cardiovascular symptoms, propranolol overdose can also cause hypoglycemia and altered levels of consciousness.

Propranolol concentrations measured in post-mortem whole blood ranged from 1490 ng/mL to 9150 ng/mL, while in urine, levels between 945 and 6500 ng/mL were detected. As the elimination half-life of propranolol is 3 to 6 h, this implies that whole blood levels of propranolol concentrations were likely higher at the time of death. Combined with the pulmonary edema, this confirms a prolonged agonal phase. In all cases, high levels of propranolol were also detected in the stomach contents. Reported plasma levels in patients who recovered from overdosing range from 1540 to 2800 ng/ml, while in autopsy cases, post-mortem whole blood levels varied between 2900 and 29000 ng/ml.

The youngest deceased had been a known drug user, presenting with ethanol in blood, vitreous humour and urine, as well as measurable concentrations of cocaine and its metabolites in blood and urine, which likely resulted in increased cardiotoxicity. In three out of the four cases, propranolol overdose was concluded to be the cause of death. In the fourth case, ischemic myocardial infarction was considered to be the cause of death. The latter was also the only case in which the deceased had taken propranolol in a therapeutic context, likely for the (secondary) prevention of ischemic heart disease. Among the four cases, the latter one also showed the lowest propranolol concentrations in post-mortem blood, urine and stomach contents.

Conclusion: All four cases presented mixed intoxication, including supratherapeutic/toxic propranolol levels measured in post-mortem whole blood. Three cases showed several intact tablets containing propranolol in the digestive system, and in all cases propranolol was detected in the gastric contents. Pulmonary edema was found in all cases, as well as elevated propranolol levels in urine samples, thus indicating a prolonged agonal phase. In three out of four cases, the cause of death was concluded to be the propranolol overdosing. The deceased presenting the lowest propranolol concentrations was the only person being treated with propranolol in the therapeutic context, and was considered to have died from ischemic myocardial infarction.

PM P-09

A review of commonly misused drugs in unnatural deaths in Cape Town, South Africa

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Background & Aims: In 2022, the Forensic Toxicology Unit of the Western Cape Forensic Pathology Service piloted a targeted UPLC-MS/MS drugs analysis at Salt River mortuary in Cape Town, South Africa. This permitted an assessment of commonly misused drugs in suspected unnatural deaths, which was previously not possible due to the backlogs at National toxicological laboratories.

Methods: A retrospective, descriptive study was conducted on all cases in the west-metropole of Cape Town for which specimens were submitted to the Forensic Toxicology Unit, between 1 January 2022 and 31 December 2022, for a targeted UPLC-MS/MS analysis for 31 common drugs. This assay was previously validated using SANAS and ASB/ANSI method validation standards. Available blood alcohol and toxicology results submitted from the same cases to the National Forensic Chemistry Laboratory were also reviewed.

Results & Discussion: The Forensic Toxicology Unit analysis was requested in 735 post-mortem cases in 2022, with qualitative and quantitative analysis performed in 723 (98.4%) and 108 (14.7%) cases, respectively. Decedents were mostly male (69.5%) and adults (30-45 years) (n=285, 38.8%), with a mean age of 31 years (SD: ±18.8). The primary causes of death were pending investigation (n=217, 29.5%), hanging (n=137, 18.6%), trauma (n=79, 10.7%), and drug toxicity (n=64, 8.7%). Drugs were detected in 382 cases (46.6%), with acetaminophen (n=179, 24.8%), methamphetamine (n=127, 17.6%), amphetamine (n=104, 14.4%), methaqualone (n=100, 13.8%) and 11-nor-9-carboxy-delta-tetrahydrocannabinol (THC-COOH) (n=93, 12.9%) most common. The National Forensic Chemistry Laboratory conducted blood alcohol analyses in 541 (73.6%) of these cases, 25.5% of which were positive (mean (SD): 0.14 ± 0.02 g/100mL; range: 0.01-0.54 g/100 mL). Toxicology results from the National laboratory were still outstanding in 89.3% (184 of 206 requests) of cases.

Conclusion: Despite a limited scope, this study presents the first comprehensive and validated data on drugs (other than alcohol) in post-mortem toxicology casework in South Africa. It demonstrates the laboratory's approach to improving turn-around times for toxicology in the province, and highlights important findings, such as methamphetamine and methaqualone detection in infants. This research sheds light on the patterns of drug misuse in the Cape Town area and provides a framework for building up quantitative data of substances in drug- or injury-related deaths. The authors recommend continued improvement and enhancement of routine toxicological testing on all unnatural deaths in South Africa, to improve our understanding of the role of drugs and toxicants in local deaths.

To test or not to test – the carboxyhaemoglobin conundrum: A case report

PM P–10

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Background & Aims: Fire and smoke inhalation deaths present a significant concern in South Africa, particularly in lower socio-economic areas with densely packed informal settlement housing made up of flammable material. Daily power outages (known as `loadshedding') together with electricity provision issues compel residents to resort to alternative, often hazardous, methods for lighting, heating, and cooking, including the burning of coals. Prior studies have highlighted the risk of carbon monoxide toxicity and ethanol impairment in fire deaths, and that autopsy findings (such as cherry-red lividity and soot in the airways) may not always align with toxicological carboxyhaemoglobin (COHb) results. Scene investigation is therefore essential in indicating whether COHb analyses are required in a post-mortem case.

Methods: This study presents a case report of a 32-year-old male who was found unresponsive in a shack (a primitive, small informal dwelling typically constructed from pieces of wood, metal, and other materials) at around 18:00, with his legs lying on the bed and his face and chest prone on the floor. There was a history of him allegedly drinking and cooking with coals. An autopsy was performed 5 days later, and key post-mortem findings included congested organs, scleral haemorrhages, and signs of early decomposition, with no fatal blunt or sharp force trauma. The suspected cause of death was recorded as `under investigation' on the death notification form, and as `positional asphyxia' on the toxicology request form completed by the forensic pathologist.

Results & Discussion: Post-mortem femoral blood specimens were submitted to the Forensic Toxicology Unit laboratory for a targeted UPLC-MS/MS analysis for commonly misused drugs and COHb analysis. The pathologist noted: "To also exclude CO poisoning, however organs not suggestive". Blood was also submitted to an external laboratory (the National Forensic Chemistry Laboratory) for blood alcohol analysis and a separate routine toxicology screen. The internal Forensic Toxicology Unit results were a COHb level of 54.0%, and delta-9-carboxy-tetrahydrocannabinol detected in blood. The National laboratory reported a blood alcohol concentration of 0.02 g/100 mL, and the toxicological screen for other drugs from the same laboratory was still outstanding. Following these results, the cause of death was determined to be consistent with carbon monoxide poisoning in combination with positional asphyxia (pending additional toxicology results).

Conclusion: The presented case emphasises the importance of the scene investigation in the local South African context, where certain toxicological analyses are not routine in every case (e.g., COHb). While classic pathological signs were not present as is typically observed in CO toxicity cases (e.g. cherry-red lividity), the COHb result was consistent with CO-inhalation from the coals in the room. This case highlights a significant problem in South Africa related to the use of hazardous lighting, heating, and cooking methods in poorer areas. Enhancing education in these communities about the dangers of burning coal indoors is essential. From a medico-legal perspective, this case demonstrates the necessity of considering scene information in navigating non-routine toxicological testing. The authors therefore recommend that COHb analysis be conducted in cases where there are coals or other burnt items (e.g., wood fires) in enclosed spaces on scene, regardless of the presence of cherry-red discolouration of tissues and/or soot in the airways at autopsy.

The gabapentinoid twins – gabapentin in the USA, pregabalin in the UK

PM P–11

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Background & Aims: Gabapentin (Neurontin®) and pregabalin (Lyrica®) are commonly prescribed drugs originally intended as anticonvulsants but now more commonly used in neuropathic pain. In the USA, gabapentin is

much more commonly prescribed than pregabalin, with the converse being true in the UK. The aim of this study is to compare concentrations of gabapentin and pregabalin in two postmortem cohorts along with a comparison of other commonly detected drug classes in each.

Methods: Only cases that underwent a comprehensive postmortem blood toxicology screen in 2023 were selected. The gabapentin cohort included all cases (USA, Canada, and UK) submitted to NMS Labs for testing. For this study, the pregabalin cohort was limited to cases that originated from the UK. Gabapentin testing was performed as part of the comprehensive screen using a custom ELSIA kit. Pregabalin was exclusively tested upon request due to the lack of commercially available screening platforms. Both gabapentin and pregabalin were quantified by LC-MS/MS utilizing the same validated procedure with a linear range of 1.0 to 150 mcg/mL for gabapentin and 0.1 to 15 mcg/mL for pregabalin. Samples exceeding the upper limits of linearity were repeated on dilution.

Results & Discussion: Gabapentin was confirmed (N=7678) in 10.7% of cases tested (N=71429) ranging in concentrations from 1.0 mcg/mL to 11,000 mcg/mL with a mean and median concentration of 18 and 8.9 mcg/mL, respectively. Pregabalin was confirmed in 7.1% (N=238) of cases tested from the UK (N=3367). Pregabalin concentrations ranged from 0.14 to 2600 mcg/mL with a mean and median concentration of 34 and 8.6 mcg/mL, respectively. Combined drug use was very common for both analytes with only 2.6% of gabapentin cases (N=207) having no other drugs present, and just one such pregabalin case. For gabapentin, the most common additional drug classes detected included opioids (71%), antidepressants (50%), stimulants (35%), benzodiazepines (32%), antihistamines (26%), cannabinoids (23%), ethanol (19%), and antipsychotics (17%). The most frequent number of additional drug classes was 3 (24%), but 43% of cases had 4 or more drug classes present. Due to the large number of gabapentin positive samples, the concomitant drug findings were evaluated across four USA regions, Canada and the UK with minimal difference detected by region (except for antihistamines which were about half as many in Canada and the UK than for the cohort overall). Except for opiates and antidepressants, combined drug use for pregabalin in the UK cohort were in a different order and with higher percentage positives than in the gabapentin cohort. These included opioids (88%), antidepressants (64%), benzodiazepines (60%), ethanol (45%), stimulants (42%), antipsychotics (26%), and cannabinoids (23%). There was no significant difference in the distribution of combined drugs when either gabapentin or pregabalin were present at concentrations below or above 20 or 25 mcg/mL, respectively, which were concentrations that the gabapentinoids were considered to be contributory to the other findings.

Conclusion: Gabapentinoids are commonly prescribed drugs. In a comparison of cohorts of gabapentin (USA, Canada and UK) and pregabalin (UK) cases, 14% of gabapentin and 17% of pregabalin cases had potentially significant concentrations present. Opiates and antidepressants were the most common additional findings. Benzodiazepines were almost twice as likely to be present in the pregabalin cohort than the gabapentin cohort which may reflect the more widespread prescribing of this drug class in the UK. Combined drug use may be of particular relevance when evaluating the potential contribution of gabapentinoids in a death investigation.

PM P–12

Sudden Unexpected Death in Infancy (SUDI): A 4-year retrospective study of toxicological findings

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Background & Aims: "Sudden unexpected death in infancy" (SUDI) concerns children aged between 1 month and 1 year whose death could not have been predicted. SUDI can be attributed to pathology or accidental causes. A number of tests are carried out to determine the cause of death, including toxicological analyses of blood and hair. In France, investigations include infant under 2 years of age. The aim of this work is to highlight the value of toxicological analyses, particularly of hair, by studying cases received from 2 reference centers in the Parisian region between 2019 and 2023.

Methods: Ethanol, methanol, isopropanol and acetone were analyzed by GC-FID. HPLC-DAD, GC-MS and LC-MS/MS screenings for general drug and illicit drugs were performed as well as an immunochemical testing. Quantification of drugs (in brief: narcotics, psychotropic drugs, cardiotropic drugs, hypoglycemic drugs, analgesics ...) was achieved by LC-MS/MS. Hair analysis was performed by LC–MS/MS. After being precisely weighed, grinded and incubated in phosphate buffer pH 5.0 in the presence of internal standards, liquid-liquid extraction was performed. The substances tested included cocaine, amphetamines, opiates, NPS, psychotropic drugs, cardiotropic drugs and

analgesics. First aid, medical examiner and autopsy reports were studied when available. The cases were classified into two main groups: those for which the cause of death was clearly determined during the investigations and those for which the cause of death remained unknown.

Results & Discussion: This study included 72 infants, (male = 43, female = 29). The median age was 3,5 months. Depending on the case, we received blood and hair samples (n=41), only blood (n=27), only urine (n=1) and only hair (n=3). Regarding blood, the most common substance found was paracetamol (n=12, 18%) at therapeutic concentrations (0.5 to 20 µg/mL), followed by lidocaine and its metabolite, MEGX (n=3, 4%), ropivacaine and prilocaine (n=1 each). Some substances have been identified in an isolated way from medical treatment prior to death (nalbuphine (n=2), morphine, midazolam as a hypnotic, laudanosine metabolite of atracurium, amiodarone) or from chronic treatment of a pathology (midazolam as antiepileptic, labetalol, nicardipine, carbamazepine). In one case THC and its metabolite, THC-COOH, were found at concentrations of < 0.1 ng/mL and 1 ng/mL respectively. Finally, in one case, toxicological analysis showed elevated carboxyhemoglobin (HbCO=47.6%) identifying the cause of death as carbon monoxide poisoning.

Regarding hair, of the 44 samples analyzed, substances were detected in 34 (77%). The most frequently found substance was lidocaine (n=25, 57%), followed by paracetamol (n=18, 41%). Other local anesthetics were also found (prilocaine (n =11, 25%), ropivacaine, (n=5, 11%) bupivacaine, (n=5). Various prescription drugs were found such as antihistamine (cetirizine (n=5) associated with hydroxyzine (n=3, 7%), pheniramine (n=3), doxylamine (n=3), oxomemazine (n=2, 4.5%), chlorpheniramine (n=1, 2%)), antalgic (tramadol (n=3) and codeine (n=2)) other psychotropic drugs (prazepam, bromazepam, pimozide, venlafaxine (n=1 each)), cardiotropic drugs (verapamil and flecainide (n=1 each)). In one case, a number of drugs that were part of the infant's medication, also found in the blood, were detected in the hair (carbamazepine, midazolam, labetalol and amlodipine). Concerning illicit drugs, cocaine was detected (n=5, 11.4%), concentrations ranging from 0.4 to 47 ng/mg, associated with BZE (0.03 to 4.8 ng/mg). Levamisole, an anthelminthic drug, frequently used as a cutting agent, was found associated with cocaine (n=2). AEME was also found (n=2) indicate an exposure to crack-cocaine. In one case, cocaine was associated with MDMA, its metabolite, MDA and ketamine. In another case, cocaine was associated with morphine. Dextromethorphan was found in one sample.

Cause of death was established in 39 cases (54%), most cases involved asphyxia during sleeping (n=17, 24%) followed by cardiac death (n=8, 11%), infectious causes (n=5, 7%) and genetic pathology (n=4, 5.5%), others cause involves drowning, food suffocation and complex multifactorial cases and in one case, carbon monoxide poisoning mentioned earlier. In cases where the cause of death was not formally identified, positional asphyxia was strongly suspected in 12 cases (17%).

Due to the porous nature of children's hair, the risk of external contamination is particularly high. Therefore, interpretation of hair result in infant is very complex. Depending on the infant's age, the presence of a substance may be of various origins: in utero exposure, breast feeding, contamination from the sweat and sebum by people close to the infant or absorption of the substance. In some cases, the concentrations found associated with the infant's young age were very much in favor of maternal cocaine consumption during pregnancy and/or during breastfeeding. These results led to the launch of a social investigation, and in some cases a judicial investigation, with the placement of the siblings in a care facility.

Conclusion: Toxicological analysis is an important part of the panel of investigations to be carried out when facing case of SUDI. Toxicological tests not only rule out any toxic cause of death, but also give an idea of the infant's environment, thanks in particular to hair analysis.

Application of an UHPLC-HRMS/MS screening using dried blood spot in post-mortem cases

PM P-13

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Background & Aims: To present a development of a toxicological screening in LC-HRMS/MS using the technique of Dried Blood Spot (DBS) and its application in forensic toxicology and more specifically in post-mortem cases.

Methods: Twenty microlitres of normal liquid whole blood (mainly from autopsies) were deposited in 2 spots (2 x 10 µL) on a special paper card (Whatmann 903). After a drying period of at least 1 hour at room temperature, the whole spots were cut out and separated for incubation: one in water and the other in methanol. After 5 minutes in an ultrasonic bath, the two phases were combined and QuEChERS salt and acetonitrile were added for extraction. A centrifugation step was performed and the supernatant was evaporated to dryness under a stream of air. The residue was reconstituted with 65 µL of the mobile phase and, after a centrifugation step, the supernatant was transferred into a vial with a reducing insert and 10 µL was injected. The chromatographic separation was performed on an Accucore Phenyl-hexyl (100 mm x 2.1 mm i.d.; 2.6 µm particle size, Thermo Fisher) with a total run time of 15.5 min. Ionisation was performed by switching between positive and negative modes. A high-resolution mass spec-

trometry using orbitrap technology was used with a resolution of 35,000 for the full MS and 17,500 for the ddMS². Molecules were identified using a ThermoFisher spectral library with a 5 ppm mass tolerance. The chromatographic and detection method was already used routinely in the laboratory for so-called "classical " analyses. Only the sample preparation was adapted to DBS. Initially, the method was developed for around sixty molecules, including benzodiazepines, antipsychotics, substitution products (methadone and buprenorphine), antidepressants and narcotics. Limit of detection (LOD) and stability on paper at two temperatures (+4°C and room temperature) were each evaluated for 7 days. Calibration models were assessed from 5 to 2,000 µg/L. This method was applied to 30 real cases, including 28 post-mortem cases in very different contexts (suicide, discovery of a putrefied body, accident, etc.). The results were compared with the combination of all classical methods used in the laboratory (LC-DAD/MS, LC-MS/MS and LC-HRMS/MS).

Results & Discussion: The detection limits ranged from 5 to 10 µg/L, except for 2 molecules (clonazepam and olanzapine) for which the LOD was set at 50 µg/L. However, all the LODs were in the subtherapeutic or therapeutic concentration range for the drugs and below toxic concentration for narcotics. The calibration models were linear for all molecules in the range 5 or 10 to 2,000 µg/L. In terms of stability assessment, all compounds except buprenorphine, clozapine and olanzapine were stable at the two temperatures. For example, at +4°C, the signal of buprenorphine decreased by about 30% in 24 hours and reached 80% in 48 hours. Although the development was carried out on around sixty molecules, the library used contains around 1,550 compounds, allowing 295 identifications in the 30 real cases using DBS, corresponding to 103 different compounds, including 65 that were not included in the original development. The combination of the three classical methods led to 331 identifications. Of the molecules not identified by the DBS method but identified by conventional methods, 4 were not in the library, and the others were not in the original development but the concentrations identified by LC-DAD/MS were below 10 µg/L (LODs not assessed by the DBS method). In terms of comparison between quantifications, preliminary studies have shown a good correlation for, for example, methadone, quetiapine and oxazepam.

Conclusion: The use of DBS in forensic toxicology analysis is beginning to grow for a number of reasons: (a) the possibility of easily obtaining a blood sample from a cadaver as well as from a living person through a finger or toe prick, (b) the reduced volume required (20 µL) to perform the same analysis as with conventional methods, which require 3-4 mL, with good sensitivity, which is very practical in certain cases such as infants, exsanguinated victims, constricted veins, etc., (c) ease of storage and transport. The application of the DBS technique to post-mortem cases in very different contexts or to living subjects demonstrates the variability in the quality of the blood used and therefore the robustness of the method. However, there is a very important issue to consider when interpreting the results obtained, namely, for example, the possible environmental contamination of the sample or by sweat, whatever the sampling site (finger, toe, etc.). Forensic toxicological analysis using DBS is very promising, but further development and research is needed to address these grey areas.

PM P–14

Postmortem distribution of MDPHP in a fatal intoxication case

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Background & Aims: The 3,4-methylenedioxy-α-pyrrolidinohexanophenone (MDPHP), is a synthetic cathinone (SC) structurally correlated to the 3,4-methylenedioxypyrovalerone (MDPV) It acts as a central nervous system stimulant and its main effects are hallucinations, paranoia, tachycardia, hypertension, chest pain, and rhabdomyolysis. In recent years, the number of intoxication cases has increased even if little is known about its pharmacokinetics properties. In particular, the postmortem (PM) distribution of MDPHP remains largely unexplored, as only two lethal intoxication cases, involving an adult man and a newborn, have been previously reported. In these reports, MDPHP levels were quantified in blood, gastric content, and urine.This study aims to describe the MDPHP PM distribution in several specimens, i.e. central and peripheric blood (CB and PB), right and left vitreous humours (rVH and lVH), gastric content (GC), urine (U) and hair.

Methods: The samples were collected from a cocaine-addicted 30-year-old whose PM interval was estimated to be 3-4 h. Autopsy examination revealed unspecific findings, i.e. cerebral and pulmonary oedema. No injection marks were observed. Toxicological analyses were performed by a multi-analytical approach: head space gas chromatography for blood alcohol content (BAC); gas chromatography-mass spectrometry (GC-MS), for the main drugs of abuse; liquid chromatography-tandem mass spectrometry (LC-MS/MS), for benzodiazepines and new psychoactive substances (NPS). Regarding NPS analysis, liquid specimens were analysed by protein precipitation with acetonitrile, while hair (proximal 0.0-1.5 and 1.5-3.0 cm) were extracted by sonication (1 h, at room temperature) with methanol. Chromatographic separation was achieved by a C18 column (2.1 ×100 mm, 1.8 µm). The acquisition was in positive ionization mode targeting the following multiple reaction monitoring transitions:

290→135, 140 m/z, MDPHP; 246→91, 140 m/z, α-pyrrolidinohexiophenone (α-PHP); 192→159, 174 m/z, 3,4-dimethyl methcathinone (DMMC); 181→148, 163 m/z, mephedrone-D3 (internal standard).

Results & Discussion: BAC was negative (0.02 g/L). MDPHP was the only psychoactive substance found in the biological fluids. It was quantified in all the specimens at the following concentrations: 1,639.99 ng/mL, CB; 1,601.90 ng/mL, PB; 12,954.13 ng/mL, U; 3,028.54 ng/mL GC; 1,846.45 ng/mL, rVH; 2,568.01 ng/mL, lVH; 152.38 (0.0-1.5 cm) and 451.33 (1.5-3.0 cm) ng/mg, hair. Moreover, hair segments were also positive for DMMC (< limit of quantification: 0.01 ng/mg), α-PHP (0.59 ng/mg, 0.0-1.5 cm; 3.07 ng/mg, 1.5-3.0 cm), cocaine (6.58 ng/mg, 0.0-1.5 cm; 22.82 ng/mg, 1.5-3.0 cm) and benzoylecgonine (1.13 ng/mg, 0.0-1.5 cm; 4.30 ng/mg, 1.5-3.0 cm). MDPHP concentrations were strongly higher than the ones reported in literature for fatal cases. For these reasons, it was hypothesized that the cause of death was due to the oral ingestion of lethal amount of MDPHP. Since CB and PB were quite similar, PM redistribution was not relevant. Differences between rVH and lVH were likely due to the partial coagulation of lVH. Hair analysis confirmed the chronic consumption of cocaine and revealed past intake of MDPHP and other SCs, i.e. DMMC and α-PHP.

Conclusion: In this paper, we described the PM concentrations of MDPHP in several specimens. To the best of our knowledge, this was the first quantification of MDPHP in VH and hair, and the first comparison between CB and PB amounts in a PM case.

Challenges and insights: Methamphetamine analysis in post-mortem putrefied human tissues in a hot climate

PM P–15

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Background & Aims: The production and distribution of methamphetamine (meth) is often associated with illegal and clandestine laboratories, posing significant challenges for law enforcement and public health efforts. Global concern is growing over meth-related fatalities, as its high potential for abuse and detrimental impact on health make it an important issue in the realm of substance abuse and addiction. This concern has notably increased in Saudi Arabia, where the hot climate adds complexity to the analysis due to challenges posed by putrefaction. There is still an urgent need to enhance the screening capabilities of many toxicology laboratories to determine the cause of death, whether it be due to drug use or natural causes.

Aim: This research aimed to investigate meth concentrations in post-mortem putrefied human solid tissues in a hot climate and comparing meth metabolite concentrations in cases where signs of putrefaction were observed versus those with no signs of putrefaction. The objective is to assist criminal investigations by analyzing meth and its metabolite concentrations.

Methods: This retrospective cohort study involved postmortem samples from human subjects during autopsies conducted between 2016 and 2022. It focused on analyzing meth and its metabolite concentrations using LC-MS/MS analysis. In this study, we analyzed various bodily fluids and tissue specimens. These included blood samples collected with sodium fluoride, vitreous humor, urine, bile, gastric contents, as well as tissues from the kidneys, brain, and stomach wall. Data on demographics, medical history, age, location, putrefaction, and other drug use were retrieved from medical records.

Results & Discussion: Results: In this study, we examined a total of 27 postmortem cases related to meth. The causes of death were either directly associated with methamphetamine or occurred in combination with other central nervous system (CNS) drugs, such as heroin. Out of the 27 reported samples of meth and its metabolite amphetamine in both putrefied and non-putrefied biological fluids and tissues, only 8 (30%) exhibited signs of putrefaction between 2016 and 2022. Despite decomposition, detectable concentrations of meth and amphetamine were sufficient to determine the cause of death and the source of amphetamines.

Conclusion: This study found no significant difference in concentrations between putrefied and non-putrefied cases, underscoring the importance of multiple sample testing during autopsy for accurate interpretation. Each case is unique and must be considered individually.

Multidisciplinary medico-legal investigation of a fatal intoxication case involving multiple traditional and novel psychoactive substances

PM P–16

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Background & Aims: Novel psychoactive substances (NPS) are an ever-growing group of heterogeneous and newly synthesized molecules, the pharmacodynamic and pharmacokinetic characteristics of which are in many cases still unknown. Furthermore, the combined consumption of traditional illicit drugs and NPS may result in unpredictable and potentially dangerous and deadly effects for users.

We present here the results of a multidisciplinary medico-legal investigation of a fatal intoxication case involving multiple psychoactive drugs, both traditional and NPS.

Methods: In this case, circumstantial, documentary, and post-mortem procedures were used, with an integrated multidisciplinary approach including on-site inspection by law enforcement officers, forensic autopsy, histopathological examination, and toxicological analysis of seized drugs, paraphernalia, and autopsy samples (peripheral supraclavicular blood and urine).

Results & Discussion: A 21-year-old man was found dead in his bedroom by a flatmate. The decedent was a university student and had a history of substance-related disorders, with previous overdoses reported. At the scene, law enforcement officers found no signs of violence to suggest third party involvement. In the subject's single room, officers found and seized several packages likely to contain drugs, along with written notes of their weighing and consumption, and numerous paraphernalia.

The most significant autopsy findings were, at the external cadaveric examination, the detection, on the left upper limb, of two puncture marks in the cubital fossa and one puncture mark on the anterior region of forearm, and, upon cadaveric section, diffusely congested lungs, markedly filled with reddish foam Histopathological analysis revealed the presence of hemorrhagic pulmonary edema and fragmentation of alveolar septa; it also showed sporadic areas of myocardiosclerosis and mild hyperplasia of the coronary artery intima, microvesicular steatosis, and brain vascular congestion with mild and diffuse perineuronal edema.

Toxicological analysis of the seized material revealed that five packages individually contained bromazolam, 2-fluoromethamphetamine (2-FMA), methoxyisopropamine (MXiPr), 3-hydroxyphencyclidine (3-HO-PCP), and 4-ethyl-2,5-dimethoxy-(N-(2-hydroxybenzyl))phenethylamine (25E-NBOH). One vial contained a solution of 4-fluoromethylphenidate (4F-MPH), and some mushrooms were found to contain psilocin and psilocybin. Analysis of paraphernalia (syringes, filters, spoons, cotton swabs) revealed the presence of heroin, cocaine and their adulterants (dextromethorphan, caffeine, paracetamol, lidocaine) and the NPS bromazolam, 2-FMA and MXiPr.

Toxicological analysis of blood allowed the qualitative and quantitative determination of carboxy-THC, cocaine and metabolites, heroin metabolites and adulterants, dextromethorphan, ketamine and metabolites, ethyl alcohol. The same drugs and metabolites were detected in urine with the addition of ecgonine methyl ester, cocaethylene and 6-monoacetylmorphine. Bromazolam, 2-FMA and MXiPr, together with many of their phase I and II metabolites, were identified by LC-HRAM-Orbitrap-MS in both blood and urine samples.

Although none of the quantified substances found in the blood was present in lethal concentrations when considered individually, given the number of substances involved in this case of polyintoxication and the synergistic and unpredictable effects they undoubtedly had, the cause of death was considered to be neuro-bulbar respiratory depression and hemorrhagic pulmonary edema induced by an accidental overdose of multiple psychoactive drugs, both traditional and NPS.

Conclusion: In drug-related deaths, a multidisciplinary approach to the medico-legal examination is extremely important, to make an integrated assessment of the autopsy and toxicological findings, in order to reconstruct the chain of pathophysiological events leading to death. The peculiarity of the present case lies in the multiple drugs found in both non-biological and biological samples, including several NPS. Understanding these events is undoubtedly a challenge, firstly to identify the psychoactive drugs involved, and secondly to assess the possible pharmacodynamic and pharmacokinetic effects of these drugs on the human body, and to apply them to the specific case, which is characterised by its own peculiarities. However, this is an important step in increasing knowledge about NPS and their possible interactions with traditional illicit drugs or psychoactive medications, and, most importantly, in preventing drug-related deaths.

PM P–17

High risk wrapping – a fatal drug mule incident: A case report

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Background & Aims: A 29-year-old male was found unconscious on a long-distance bus traveling from the Netherlands to Poland. Upon arrival of paramedics, the patient was initially resuscitated from asystole for 70 minutes.

Unusual skin discoloration and detachment of the belt area was notable. There was a leaking foil packaging containing an unknown liquid of pungent, irritating odor attached to the chest and pelvis. The cause of death was undetermined, and an intoxication was suspected.

Methods: Toxicological analyses of femoral blood, urine and stomach contents of the deceased were performed using liquid-chromatography-tandem and liquid-chromatography-quadrupole-time-of-flight mass spectrometry, respectively. The unknown liquid was analyzed using Raman spectroscopy, capillary gas chromatography combined with mass spectrometry, and nuclear magnetic resonance spectroscopy.

Results & Discussion: In addition to low concentrations of THC and its metabolites as well as traces of benzoylecgonine, ecgonine methyl ester, and lidocaine, the deceased had an amphetamine concentration of 60,000 ng/mL in the femoral blood. The unknown liquid was identified as amphetamine oil, with an active ingredient content of 57.2 %. Amphetamine oil can slowly penetrate plastic materials, such as the foil packaging in the present case. After skin contact, the acid mantle of the skin is deactivated, causing the protocolled skin discoloration, detachment, and chemical burns. As soon as the skin is damaged, amphetamine oil is readily absorbed through the skin.

Conclusion: The cause of death can be attributed to an amphetamine intoxication due to dermal absorption from the leaking packaging.

Analysis of ethyl glucuronide and ethyl sulphate in post mortem cases: can alternative matrices be used?

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Background & Aims: Caution must be shown when interpreting blood-alcohol concentrations in post mortem cases due to the possibility of post mortem formation of ethanol. The biomarkers ethyl glucuronide (EtG) and ethyl sulphate (EtS) are used to discern between ante mortem ingested ethanol and post mortem formed ethanol. If ethanol, EtG and EtS are detected, this strongly support ante mortem ingestion of ethanol, while positive ethanol in the absence of EtG and EtS indicates that ethanol is formed post mortem. Analyses of EtG and EtS for this purpose are preferably performed in peripheral blood, but this is not available in for instance heavy putrefied and burned cases. The aims of this study were to investigate if post-mortem formed ethanol was found in different matrices, and to study the distribution of EtG and EtS after ante-mortem ingestion of ethanol.

Methods: The results from 46 autopsy cases with findings of ethanol are presented. Peripheral blood, cardiac blood, pericardial fluid, psoas and lateral vastus muscle, and vitreous humour were collected from each case, with a few exceptions where not all matrices were available. Ethanol was screened and quantified by a headspace gas chromatography flame ionization detector method (HS-GC-FID). EtG and EtS were quantified by UHPLC-MS/MS methods. Concentrations in the different matrices were compared with the corresponding concentration determined in peripheral blood.

Results & Discussion: In five of the ethanol positive cases, neither EtS nor EtG was detected in any matrices, and these cases were interpreted as post mortem formation of ethanol. Ethanol was detected in pericardial fluid and psoas muscle in all these five cases. In psoas muscles the concentrations of ethanol were high, and the highest levels were 1.6 and 2.1 g/kg. Ethanol was also detected in cardiac blood in the three cases where this was available, whereas there were both positive and negative findings of ethanol in lateral vastus muscle and vitreous humor. It is not known if detection of ethanol in these cases was due to post mortem formation in the different matrices, or distribution of ethanol between the matrices.

In 41 of the ethanol positive cases, EtG and EtS were detected in peripheral blood and this was interpreted as ante mortem ingestion of ethanol, although post mortem formation in addition could not be excluded. EtS and EtG were detected in the corresponding cardiac blood and pericardial fluid samples of all cases, but in psoas muscle, EtS and EtG was detected in 32 cases (78%), and in vastus lateralis muscle, EtS and EtG were detected in 84% of the cases. In vitreous humor, EtS and EtG were detected in 32 cases (78%).

Conclusion: In cases with post mortem ethanol formation, ethanol is likely to be detected in several matrices. If peripheral blood is not available, our results revealed that EtG and EtS are likely to be detectable in cardiac blood and pericardial fluid after ante mortem ethanol ingestion. In muscle tissue and vitreous humor, however, EtG and EtS results should be interpreted with more caution and negative results does not necessarily represent post mortem formation of ethanol. Although EtS is previously shown to be stable, more research is needed regarding stability in post mortem muscle tissues.

PM P–19

Determination of oleandrine in postmortem organ tissues of two acute oleander poisoning victims by LC-MS/MS

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Background & Aims: The oleanders are evergreen flowering shrubs that grow well in the southern region of South Korea. The oleanders contain various cardenolides, a sort of cardiac-active steroid, which have inotropic effect on the hearts of animals and humans. The most toxic effect of oleanders is due to oleandrine, a cardiac glycoside isolated from the leaves, flowers, and seeds. Oleandrine has been used for heart stimulant and diuretic, but has been associated with many fatal outcomes. Mother and daughter, ages 73 and 46 were found dead together in their house leaving a suicide note. In an autopsy to reveal the cause of death, liver, kidney, and gastric tissues were collected and tested for toxicological analysis. Blood sample collection was not possible due to severe decomposition of the corpses. The toxicological results showed that oleandrine was detected in these postmortem tissues.

Methods: Liquid chromatography-tandem mass spectrometry (LC-MS/MS) method with solid-phase extraction (SPE) was developed and validated for the analysis of oleandrine in organ tissues. For the ionization conditions, an analysis in the ESI positive mode was conducted.

Results & Discussion: The validation parameters including linearity, accuracy, precision, matrix effect, and recovery were satisfactory. The correlation coefficients (r2) of the liver tissue was 0.995 in the concentration range of 5-500 ng/g. The concentrations of oleandrine in liver tissues of mother and daughter was 0.60 mg/kg and 1.67 mg/kg, respectively. Oleander plant was found in the house but not collected because police officer was unware of the plant. The cause of death was determined to be acute fatal intoxication with oleandrine. This is the first case of quantitation of oleandrine in decomposed bodies in South Korea.

Conclusion: In this study, we developed and validated a method that combined SPE and LC-MS/MS. We performed a quantitative analysis of oleandrine in postmortem organ tissues from two oleander poisoning victims. Our findings confirmed that this method was sensitive and selective in the detection and quantification of oleandrine in organ tissue samples. The developed analytical method would be beneficial in the clinical toxicology and forensic toxicology fields.

PM P–20

Cocaine and alcohol use and its relationship with homicidal deaths

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Background & Aims: Unintentional and violence-related injuries account for 4.4 million deaths worldwide; for those aged between 5-29 years, road traffic crashes, homicides and suicides are three of the five main manners of death. The study of the causes of intentional and unintentional deaths is not an easy task, especially when it considers only the victims. However, the use of alcohol and other psychoactive drugs play a significant role for victimization on those cases. Stimulants such as cocaine may increase the odds for injuries and deaths. High homicidal death rates are a fact in several countries and drug use may contribute significantly with those numbers. Therefore, the aim of this study was to evaluate the influence of cocaine and alcohol use on cases of homicidal deaths.

Methods: We followed the methodology described by Andreuccetti et al., 2016. Postmortem samples were collected during autopsy in four Brazilian cities. The sampling strategy allowed us to obtain a representative sample of all violent death cases occurring in each day of the week of each involved cities. We sampled all days and hours in a similar proportion from March/2022 to September/2023. We included all autopsied older than 18 years, with less than eight hours from the fatal injury. Cases with more than eight hours after death, in advance stage of body decomposition, victims who received six or more hours of medical treatment prior to death were not included. We collected either cardiac or peripheral blood in tubes containing sodium fluoride. All samples were analyzed by liquid chromatography-tandem mass spectrometry, for the presence of THC, cocaine, and its metabolites (benzoylecgonine, cocaethylene, and AEME), amphetamine, methamphetamine, MDMA, MDA and benzodiazepines (alprazolam, diazepam, clonazepam, oxazepam, bromazepam, and 7-aminoclonazepam). Results for benzoylecgonine, major cocaine metabolite, were used to assess cocaine use. Blood alcohol concentration was determined by gas chromatography-FID. This study received founding from the Nacional Secretary of Drug Policies of the Brazilian Ministry of Justice and Public Safety.

withdrawn

Results & Discussion: We included 3,355 deceased victims and nearly half of them (47.8%) had consumed at least one psychoactive substance before fatal event. This percentage increased to 54.7% on homicide cases, which accounted for 1,769 cases of all causes of death. Victims of homicides had consumed psychoactive substances in

a larger proportion in comparison to victims of other causes of death (i.e. traffic crashes, suicides) (54.7% vs 40.0%, p<0,01). The consumption of alcohol did not present a statistical significance difference on victims of different manners of death (26.3% for homicides and 24.2% for other causes). However, homicide victims consumed cocaine more than three times of other victims (35.4% vs 11.6%, p<0,01). The detection of AEME (crack cocaine biomarker) was also higher in homicide victims (1.6% vs 0.8%). More than 82% of the homicides were gun related. However, cases of injuries due to sharp-force trauma (i.e. knife-related), presented the highest prevalence of substances detection (76.6%), mostly alcohol (57.1%), and one third had consumed cocaine before fatal injuries. For the gun-related cases, cocaine was the most consumed substance (35.4%). In one hand it is difficult to study the victimization and influence of drug use on homicide cases. On the other hand, massive literature data point that stimulant drugs may increase the odds for involvement in events presenting higher risks for injuries and deaths. Accordingly to Goldstein's conceptual framework, the pharmacological effect of drug use is one of the aspects explaining the drug-related violence. Indeed, the results found by the present study corroborate that cocaine may play a major role in victimization due to homicides, or at least, may increase the odds for fatal injuries, once frequence of cocaine use among victims from manners causes of death (e.g. suicidal and traffic-related) was far lower than for homicidal victims. Although the correlation between cocaine consuption and death was measured trough the detection of its metabolite, benzoylecgonine (BZE), the results indicate acute drug use. Cocaine has rapid metabolism, quickly converting into BZE after consumption, and such hydrolisis can even occur in vitro. Therefore, the results demonstrate that either the victim was under the influence of cocaine or had consumed it shortly before the fatal event. In this sense, our findings confirm a strong correlation between homicides and cocaine use by the victim. Furthermore, these findings could aid in comprehending victimization in homicides and in reducing the exceedingly high rates of such crimes. Implementing public policies aimed at reducing harm related to the use of psychoactive substances could potentially bring down these numbers.

Conclusion: More than one in each two victims of homicides had consumed psychoactive substances before death. Cocaine was the most consumed drug and more than one third of the victims used it. Alcohol was the second most used drug. Gun-related victims consumed more cocaine than other homicide victims and cold weapon-related homicides had a higher detection of alcohol. In fact, cocaine seems to play an important role on the victimization by homicides.

Preventing the undetected murder: Practicable and rapid ICP-MS method for the detection of thallium, lead, and arsenic in post-mortem blood

PM P-21

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Background & Aims: Homicide, suicide or accident - elemental intoxication may be a cause in each of these types of deaths. Inductively coupled plasma mass spectrometry (ICP-MS) has emerged as the gold standard analytical method for toxic metal analysis. The aim of this project was to develop a practicable and rapid quantitative method for the determination of arsenic, lead and thallium in post-mortem blood samples, whereby a clinically validated method was to be adapted for forensic purposes.

Methods: An ICP-MS method was developed using a modified acidic work-up for the quantitative determination of arsenic, lead and thallium. The method was optimized for post-mortem blood matrix using commercial a whole blood calibrator and controls; only 200 µL of blood is required. Method validation focused on the assessment of linearity, between- and within-day precisions, limits of detection (LoD) and lower limits of quantification (LLoQ), and carryover. The method was applied to retrospective analysis of 279 post-mortem peripheral blood samples.

Results & Discussion: Using 6-point and 1-point calibrations, precisions and accuracies ranged from −4.8 to 5.8% and −6.4 to 7.5%. Analytical sensitivities for As, Pb and Tl were 0.08, 0.18 and 0.01 µg/L (LoD) and 0.23, 0.66 and 0.03 µg/L (LLoQ), respectively. Observed post-mortem peripheral blood concentrations were: As, 1.31 ± 3.42 µg/L; Pb, 17.4 ± 13.1 µg/L; Tl, 0.11 ± 0.07 µg/L (mean ± standard deviation (SD)). Elemental concentrations, determined in additional quality controls samples, were in good agreement to those obtained with an external ICP-MS method based on alkaline sample processing.

Conclusion: The current method is rapid and practicable as well as compatible with an ICP-MS system used for trace element analysis in an accredited medical laboratory. It serves as a foundation for implementing routine low-threshold investigations of metal intoxications in post-mortem blood and can be included in routine forensic diagnostics.

PM P–22

A case report of a fatal levomepromazine overdose with unusual concentration

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Background & Aims: Levomepromazine (methotrimeprazine) a phenothiazine neuroleptic renowned for its sedative effects, plays a vital role in palliative care and psychiatric treatment. Despite its therapeutic advantages, the drug is associated with side effects, including an escalated risk of sudden cardiac death at higher doses. This case report sheds light on an unprecedented post-mortem concentration of levomepromazine, surpassing documented levels in existing literature. The primary objective is to heighten awareness and contribute to bolstered patient safety within psychiatric hospital settings.

Methods: Data collecting from autopsy case report, law enforcement decree and toxicology report. Regrettably, critical specifics pertaining to the methodologies were not conveyed to our team. Furthermore, it appears that the communication from the judicial laboratory focused solely on relaying the results deemed pertinent to their investigation.

Results & Discussion: A man in his thirties, undergoing psychiatric treatment, was discovered unresponsive in his isolated room. Autopsy findings, 36 hours post-mortem, revealed superficial skin depressions resembling window bars on the scalp, neck, and knees, devoid of internal traumatic lesions or underlying pathologies. Post-mortem specimens, including femoral blood samples and gastric contents, were collected and promptly sent to the judicial toxicology laboratory for analysis. The analysis of blood and gastric samples yielded results indicating the presence of two neuroleptic substances. Levomepromazine was detected in a notably high concentration at 11059 ng/ml (11,059 mg/L), whereas chlorpromazine exhibited a normal concentration of 2405 ng/ml (2,505 mg/L). The concentrations of both substances in gastric contents were not measured. The results outlined above represent the exclusive data available in the toxicology report, which was forwarded to us by the judicial laboratory. The cause of death was identified as a fatal levomepromazine overdose during hospitalization. Investigation into the circumstances facilitating the patient's access to this medication is ongoing, with details pending.

Conclusion: In conclusion, the distinctive and significantly heightened level emphasizes the seriousness of our case, underscoring the imperative for a thorough investigation into the circumstances that led to such an extreme concentration. While levomepromazine plays a crucial role in psychiatric care, its potential for toxicity underscores the significance of vigilant prescribing practices, close monitoring, and patient education. Delving into the factors contributing to fatal overdose incidents, as exemplified in the current case, is crucial for enhancing safety protocols and safeguarding the well-being of patients undergoing psychiatric treatment in psychiatric hospitals.

PM P–23

Death in children after *Atractylis gummifera l*. poisoning in Morocco—report of three cases and review of literature

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Background & Aims: Atractylis gummifera L. is a poisonous thistle plant that grows in the Mediterranean regions especially in northern Africa like Morocco and southern Europe. It has been used frequently to treat some diseases in traditional medicine, and its ingestion is a common cause of fatal poisoning. Here, we report 3 death cases in children after accidental ingestion of the Atractylis gummifer L.

Methods: Data collecting from autopsy reports, toxicology reports and law enforcement investigation data. The methods utilized in this study encompassed several analytical techniques for the extraction, identification, and qualitative analysis of toxic compounds, particularly Atractylis gummifera L (ATR), in postmortem biological samples. Initially, root extraction and identification were conducted using thin-layer chromatography (TLC) and high-performance liquid chromatography coupled with a diode array detector (HPLC/DAD). Blood and gastric fluid samples were then prepared by mixing with internal standards and acetonitrile, followed by derivatization at 100°C. Subsequently, three methodologies were employed for sample analysis: TLC, HPLC/DAD, and gas chromatography-mass spectrometry (GC-MS). TLC involved impregnating a chromatography plate with the extract and standard, with elution using a ternary phase and development under iodine vapor. HPLC/DAD utilized a C18 column with detection at 195 nm, while GC-MS employed negative ionization mode with quantification performed on [M-H]- and [M-H + 1]- ions of the ATR molecule. However, quantification via GC-MS was deemed unreliable due to fragmentation variability, rendering all methods qualitative only. The toxicology qualitative analysis revealed the presence of ATR in the blood and gastric fluids of all cases.

Results & Discussion: We report 3 cases of death in children after accidental ingestion of the poisonous plant Atractylis gummifer L. The poisoned children were admitted to hospital in deteriorated general state with clinical

symptoms, such as nausea, vomiting, epigastric, and abdominal pain, diarrhea, followed by coma. However, they died a few hours later. The postmortem investigations were performed, and the diagnosis of Atractylis gummifer L. poisoning was confirmed by toxicological examination (Chromatographic system HPLC/DAD). We utilized a HPLC type 'Waters, alliancer' with a separation module (2695), an automatic injector: Model SM7 (capacity 100 µL); a DAD (UV 2696) and controlled by 'Waters, Empower Pro' software. The column used is a C18 'X-terra' 150 mm. The absorption wavelength is 195 nm. The retention time of ATR is 3.23 minutes, with a relative standard deviation of 1.23. Analysis time is 5 minutes. The detection system employed was a diode array detector (DAD).", the latter showed the presence of atractyloside (potassium atractylate), a toxic compound of the plant Atractylis gummifera L. Atractylis gummifer L.

Quantification was performed on the [M-H]- and [M-H + 1]- (isotopic contribution of 13C) ions of the ATR molecule to maximize the signal-to-noise ratio: the noise signal for the 13C isotopomer of the molecule was zero, and adding the intensity of the ions enabled us to artificially increase the signal of the ATR without increasing the noise. Gas chromatography–mass spectrometry was performed at high-collision energy to achieve the loss of a sulfate group from the [M-H]- ion of ATR (725 > 645 amu [M-H-SO3]-) and start the fragmentation of these daughter ions to produce a rich fragmen- tation spectrum. However, the spectra obtained were not informative enough to enable the identification of the ATR compound. On the other hand, the fragmentation lacked repeatability, which invalidated the method for quantification. Thus, we used SIM, the full-scan product ion spectrum of the ATR compound only to confirm the identity of the toxin. So no quantification was done.

All methods were qualitative only, and the results of toxicology qualitative analysis of the postmortem biological samples showed the presence of atractyloside (potassium atractylate), a toxic compound of Atractylis gummifera L plant, in blood and gastric fluids of all cases. Poisoning was discussed with review through the literature.

Conclusion: Through the presented cases, we show that Atractylis gummifera L. poisoning remains a health problem that involves children in Morocco, where the plant grows spontaneously. Thus, teaching children to recognize dangerous plants will be helpful to prevent accidental ingestion.

Determination of tramadol and its metabolite O-desmethyltramadol in blood and vitreous humor samples

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Background & Aims: Tramadol is a synthetic centrally acting analgesic with atypical opioid action, while its active metabolite O-desmethyltramadol is involved in the opioidergic mechanism and exerts the analgesic activity of the parent drug. Blood levels of tramadol higher than 1 mg/L are considered toxic, and may indicate contribution to the cause of death, while many articles have reported death of individuals due to tramadol alone at concentrations higher than 1.6 mg/L. Furthermore in recent years, there has been increasing interest on the use of alternative biological materials in post-mortem forensic toxicology. Vitreous humor is one of them, which, due to the closed cavity where contained, has a low degree of contamination and high purity that makes it ideal during toxicological investigation of forensic cases with post-mortem samples. The aim of this study was the development and validation of a gas chromatography/mass spectrometric (GC/MS) method for the determination of both tramadol and O-desmethyltramadol in vitreous humor and blood samples and its application to real biological samples from forensic cases in order to investigate the distribution of the two substances in this alternative biological fluid.

Methods: A GC/MS method was developed, validated and applied to post-mortem blood and vitreous humor samples obtained from 12 forensic cases. The presence of tramadol in these samples was confirmed during general screening of blood or urine samples or the intake of tramadol had been reported in the case history. The sample preparation procedure included solid-phase extraction and derivatization using N,O-Bis(trimethylsilyl)trifluoroacetamide with 1 % trimethylsilyl chloride prior to GC/MS analysis.

Results & Discussion: The method was fully validated according to international guidelines (FDA, ICH and SWGTOX). For both analytes, the LOD and LOQ were 1.50 and 5.00 ng/mL, respectively. The calibration curves were linear (R2 ≥ 0.992) from 5.00 to 1000.0 ng/mL (dilution factors were also validated when > 1000 ng/mL), and absolute recoveries were higher than 85 %. Accuracy and precision were within the accepted range (±15 % for QC and ±20 % for LOQ concentration). Both substances were found to be readily distributed in vitreous humor, since even in cases of very low concentrations of the analytes in blood, their detection was also possible in vitreous humor. The blood concentrations of tramadol and O-desmethyltramadol ranged from 136.8 to 1888 ng/mL and from 5.6

to 239.3 ng/mL, respectively. The respective vitreous humor concentrations were found to be for tramadol from to 58.4 to 1254 ng/mL, and for O-desmethyltramadol from 5.2 to 287.1 ng/mL. In addition, the ratios of vitreous humor concentrations to the respective blood concentrations were calculated in order to study the distribution of tramadol (0.43-2.29) and O-desmethyltramadol (0.53-2.16) in the vitreous humor. Finally, the mean and median ratios of vitreous humor to blood concentrations for the two substances were also calculated. The mean values were found to be 0.91 and 0.94 for tramadol and O-desmethyltramadol, respectively, while the median values for tramadol and O-desmethyltramadol were 0.75 and 0.83, respectively.

Conclusion: The study of the above post-mortem samples shows the importance of using vitreous humor as an alternative biological material either in cases where blood and urine samples cannot be collected or for drawing safer conclusions since vitreous humor is much less affected than blood by the phenomenon of sepsis and post-mortem redistribution. However, in order to establish therapeutic and toxic concentrations of tramadol and its metabolite in vitreous humor, corresponding to those in blood, further analysis of biological samples from a larger number of forensic cases, and in particular cases of overdose, seems necessary.

PM P-25

A case of chemsex with the presence of 3-chloromethcathinone (3-CMC)

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Background & Aims: clophedrone, or 3-chloromethcathinone, or 3-CMC, is a synthetic cathinone which first appeared in 2014 and then more regularly detected since 2020, and for which few data are available. We present here a case of chemsex involving 3-CMC. We report the case of Mr X, aged 62, who was found dead in his home, naked and face down. No resuscitation was carried out. Found at his side were three sachets of white powder, two syringes, one full, the other almost empty, a vial labelled "Iron Fist" and two computer screens. All this suggests that he had practised chemsex, probably alone according to the investigators. The autopsy performed 48 hours later revealed non-specific diffuse polyvisceral congestion, cardiomegaly and recent injection marks on the left arm. The usual anatomopathological and toxicological samples (fluorinated peripheral blood, cardiac blood, gastric contents and urine) were taken. in this context, the use of cathinones was suspected. Because of the instability of these molecules, a sample of fluorinated peripheral blood was frozen as soon as the samples were received in the laboratory. The powder, syringes and vial found at the scene, were seized.

Methods: Peripheral blood stored at +4°C was analysed for alcohols by HS-GC/FID, HbCO by spectrophotometric method, drugs and narcotics by GC-MS and LC-HRMS, and GHB by GC-MS. Cannabinoids were detected using a specific LC-MS/MS technique. Cathinones were also detected by LC-HRMS in frozen fluorinated peripheral blood (-80°C), as well as in urine and gastric content stored at +4°C. The seizure products (powders, syringe contents, vial) were analysed by LC-MS/MS using 3- and 4-CMC standards. The vial was analysed by GC-MS.

Results & Discussion: Analyses of peripheral fluorinated blood revealed a physiological level of HbCO, a blood alcohol level of 0.06 g/L, a toxic concentration of GHB of 330 µg/mL and the presence of dolutegravir and rilpivirine at therapeutic concentrations (623 and 74 ng/mL respectively). The determination of cathinones in the frozen blood sample showed the presence of x-CMC at 17 ng/mL, while no cathinones were identified in the blood, urine and gastric content samples stored at +4°C. Separation of 3- and 4-isomers in peripheral blood was impossible due to the lack of chromatographic resolution in LC-HRMS. Poppers are not routinely tested.

LC-MS/MS analysis of the powder with the two 3- and 4-CMC standards confirmed the presence of 3-CMC with a concentration close to 100%. The syringe also contains 3-CMC (240.7 mg/mL for a volume of 600 µL). Analysis of the vial labelled "Iron Fist" by GC-MS shows the presence of amyl nitrite and isoamyl alcohol. Neither GHB nor a precursor was detected in the seizure products, although the very small volume contained in the second syringe meant that an exhaustive search was not possible.

Pathological examination revealed mitral valvulopathy, microinfarct lesions and acute emphysema, suggesting a mechanism of asphyxia with probable positional involvement.

The profile of substances identified (GHB, cathinone, poppers) confirms a chemsex practice, although cocaine or amphetamines, regularly associated with cathinones and GHB, were not found in this case. The recent injection marks on the left arm are consistent with slamming. It should be noted that dolutegravir and rilpivirine do not correspond to either PrEP (pre-exposure prophylaxis) or PPE (post-exposure prophylaxis), but probably to the victim's long-term antiretroviral treatment, although no information is available regarding his serological status. According

to the pathologist, death probably resulted from positional asphyxia secondary to a disturbance of consciousness induced by a toxic concentration of GHB. The few data in the literature concerning 3-CMC seem to support recreational use (Pieprzyca E. et al. J Anal Toxicol 2022;46:1008-1015). However, given the instability of 3-CMC, confirmed by its absence in samples stored at 4°C, it is impossible to formally rule out the possibility that the initial concentration was higher and may therefore have contributed to the death. Finally, the possible involvement of poppers, if not confirmed by blood analysis, should also be considered.

Conclusion: All in all, this case confirms the appearance of 3-CMC on the NPS market, and once again highlights the instability of cathinones in biological samples.

Silent witnesses: Non-biological samples as keys to postmortem toxicological analysis

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Background & Aims: Most forensic toxicology casework is centered around the analysis of biological matrices (e.g., blood, urine and vitreous humour). However, non-biological samples (e.g., powders, unidentified pills, suspicious bottles) sometimes hold the keys to an efficient and fruitful method selection for biological material. Three such postmortem cases are presented here, where non-biological samples' analysis revealed information orienting the toxicologist towards specific and relevant analyses for the standard biological samples collected.

Methods: All biological samples were analyzed by a wide-scope targeted method quantifying 83 and identifying 116 xenobiotics (parent compounds and metabolites). Samples were extracted by protein precipitation with a 7:3 acetonitrile:acetone mixture, and the diluted supernatant was analyzed on an Agilent 1200 HPLC coupled to a Sciex 5500 QTrap in positive MRM mode (LC-MS/MS).

Non-biological samples were submitted to gas chromatography – mass spectrometry (GC-MS) general unknown screening. Such a method is more time consuming but covers a wider range of xenobiotics than the targeted method. It is also less sensitive than an LC-MS/MS method, which is typically not an issue with non-biological materials. Generally, samples were prepared using a simple dilution of the material. If needed, a solid phase extraction preparation (Oasis HLB cartridges) was also used prior to instrumental analysis.

Analysis of nitrite/nitrate was performed by NMS Labs (blood test 3174B).

Results & Discussion: In the first postmortem case, findings from a non-biological sample modified the results reported for biological samples. Indeed, analysis of the femoral and cardiac blood, urine and vitreous humor revealed the presence of gamma-hydroxybutyrate (GHB) along with several other substances. However, concentrations in central blood (16 mg/L), femoral blood (16 mg/mL) and vitreous (23 mg/L) were fairly low for a postmortem case – urine alone showed higher levels of GHB, with an estimated concentration of 250 mg/L. Postmortem GHB neoformation being a well-known phenomenon, and with urine alone clearing the 50 mg/L threshold (analytical cutoff set at 9 mg/L), GHB presence in this case would have been conservatively interpreted as a putrefaction artefact. However, police officers had seized a suspicious bottle among material from the scene and sent it to be analyzed for drug chemistry on behalf of the coroner. The positive result for GHB was later shared with the forensic toxicologist assigned to the case. This confirmation of GHB consumption lessened the probability that the analytical findings were attributable to neoformation, and GHB was added to the toxicology report.

In the second case, observations on the scene oriented the selection of an unusual, targeted analysis method for the biological samples. A young adult was found dead on his bathroom floor; yet external examination revealed no potential cause of death. Fortunately, police officers on the scene noticed an envelope identified "Sodium Nitrite" and reported this to the coroner who included it in his toxicological analysis request. Femoral and central blood samples were therefore sent for nitrate/nitrite analysis, even though the distinctive brownish colour related to blood oxidation by nitrite was not observed at autopsy. Levels compatible with an intoxication (>1000 µmol/L) were found. Toxicological results of biological samples were otherwise unremarkable. Without information about the non-biological item, the probable cause of death would have likely been missed.

In the last case, non-biological items oriented the screening strategy for biological samples. Along with several items collected on the scene, a green powder was submitted for toxicological analysis together with central and femoral blood. General unknown screening by GC-MS led to the identification of desalkylgidazepam, a new psychoactive substance detected for the first time in the province only a few months earlier. Consequently, blood was analysed by GC-MS, but desalkylgidazepam was not detected, possibly because of the poor sensitivity for this compound (~ 250 ng/mL). This detection failure prompted the undergoing addition of desalkylgidazepam to the targeted screening LC-MS/MS method applied on all biological samples handled by the laboratory, with a target sensitivity of approximately 0.25 ng/mL.

Conclusion: This case series emphasizes the importance of considering the information provided by non-biological samples found on the scene. This can be identification of drugs by a third party (e.g., drug chemistry laboratory); visual identification of drugs, containers, or paraphernalia; or even in-house analysis results of non-biological items. In any event, all parties involved (e.g., police officers, drug chemistry laboratory, coroner, forensic toxicologist) need to understand the importance of non-biological material and commit to fluid communication of information to other partners. These cases show once again that forensic toxicology cannot be successful as a black box, blind to all information from the scene. At a bare minimum, knowledgeable trier of fact must ensure relevant information is transmitted to the forensic toxicologist to ensure a complete death investigation is performed.

PM P-27

Micro-QuEChERS extraction combined with LC-QTOF-MS analysis for comprehensive screening of New Psychoactive Substances in postmortem blood samples

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Background & Aims: Analysis of New Psychoactive Substances (NPS) remains challenging for many institutions due to the ever-changing landscape of the drug market, where new and existing substances continuously emerge and disappear. This dynamic environment complicates the timely adaptation of analytical methods. The rapid emergence of numerous NPS globally poses significant risks to public health. Identifying and analyzing a diverse array of substances concurrently present in drug markets is inherently demanding, particularly given the increasing incidence of acute poisoning cases and deaths associated with NPS. Accurate and reliable drug testing methods are necessary to detect and confirm a wide range of analytes with varying chemical structures. This diversity represents challenges for clinical and toxicological laboratories striving to maintain updated drug screening methods. The aim of this research was to develop and validate a sensitive screening method for detecting multiple NPS classes, including synthetic cannabinoids, synthetic cathinones, fentanyl analogues, phenethylamines, designer benzodiazepines, and other psychoactive compounds such as MDMA, ketamine, LSD, and their metabolites in post-mortem blood samples. This was achieved utilizing micro-QuEChERS, a green analytical toxicology alternative technique for sample preparation, and Liquid Chromatography-Quadrupole Time-of-Flight-Mass Spectrometry (LC-QTOF-MS).

Methods: Aliquots of 450 µL of postmortem blood samples were fortified with 50 µL of the working standard and internal standard solutions. Then, 1500 µL acetonitrile was added, along with 500 mg NaAc:MgSO4 (1:4, w/w) partitioning salts, followed by agitation at 2500 rpm for 5 min and centrifugation at 3500 rpm for 10 min. The supernatant was evaporated, resuspended in 100 µL of methanol, and 5 µL was injected into a Nexera HPLC chromatographer coupled to an LCMS9030 quadrupole-time-of-flight (QTOF) mass spectrometer (Shimadzu, Kyoto, Japan) equipped with an ESI ionization source operating in positive mode. Chromatographic separation was achieved using a RaptorTM biphenyl column (100 × 2.1 mm, 2.7 µm), maintained at 40 °C with gradient elution (mobile phases: 0.1% formic acid in water and 0.1% formic acid in methanol at a flow rate of 0.3 mL/min, 17-minute run). Mass spectra were obtained using Data-Independent Acquisition (DIA) and analyzed using Insight Explore software. Both full scan and MS2 events were employed with an inclusion list containing the exact mass, molecular formula, fragments ions, and retention time of targeted compounds. Acquired fragment spectra were matched to the HighResNPS spectral library.

Results & Discussion: The proposed method enabled effective separation and identification of the target compounds. Most analytes were detected within acceptable limits of detection (≤ 1 ng/mL), with the limit for each analyte assessed and estimated. Confirmation criteria were achieved considering (I) a precursor mass error of less than 5 ppm, (II) at least 2 fragments with a mass error of less than 5 ppm in MS2 spectra, (III) a retention time within ± 2 % of controls average retention times, and (IV) a library reverse score exceeding 70%. This approach, utilizing DIA, ensures high confidence in the identification of the analyzed substances, with sensitivity and robustness, allowing unrestricted retrospective analysis of samples. Although the method was developed for targeted compounds, there is wide diversity of designer substances newly produced capable of expanding our spectral library. The continuous enhancement in substance detection allows for broad coverage of molecules, further improving its utility in forensic investigations and toxicological analysis.

Conclusion: The present study demonstrates the efficacy of micro-QuEChERS extraction associated with the DIA mode in LC-QTOF-MS analysis as an excellent screening procedure for sensitive detection of NPS in postmortem blood samples. This extraction technique facilitated rapid and reliable identification, underscoring its utility in forensic and toxicological investigations. Currently, our analysis includes over 130 compounds, with the potential for further expansion using the HighResNPS library. The versatility of this method presents promising opportunities for ongoing updates with additional compounds, thereby enhancing its capabilities in NPS detection. These findings significantly contribute to the advancement of analytical methodologies in forensic science, improving public safety and facilitating comprehensive investigations.

The forensic toxicokinetics in alcohol rated case identification in China – case study

PM P–28

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Background & Aims: The appraisal of alcohol related cases is a global challenge, which includes how to estimate the alcohol concentration in corpse at the time of death when there were a PMR, decomposition degradation or no blood), how to estimate the alcohol concentration in living body when the poisoning or accident happened, how to distinguish the postmortem production of poison and antemortem taken alcohol, how to identify antemortem taken alcohol and postmortem given alcohol and how to determine the time, dose of drinking. Previously, we proposed applying forensic toxicokinetic of alcohol and its metabolites to address the aforementioned issues. This study was to summarize and address the challenges for alcohol rated cases in the five areas mentioned above, supported by two grants from NSFC(No. 82130056 and 82072116).

Methods: Based on the forensic toxicokinetics (toxicokinetics, postmortem distribution, postmortem redistribution, postmortem diffusion, toxic decomposition kinetics) of alcohol in Chinese, through six typical cases in China, we summarized and explored the challenges in above five aspects for alcohol related cases.

Results & Discussion: For example 1, the alcohol concentration (mg/100mL or g) detected in a corpse in a fighting case with a cut carotid artery were 730.3, 159.3, 1336.6, 1212.5, 530.7, 2711.7, 1320.8, 332.4, 547.0, 205.75, 3671.7, 2022.5, 247.2, 302.0, in heart blood, vitreous humor, liver, kidney, lung, spleen, small intestine, brain, heart, testis, stomach content, gastric wall, calf muscles, chest muscle , and EtG (ng/mL) were detected as 5012.5 and 1053.7 in heart blood and vitreous humor, EtS (ng/mL) were as 1392.5 and 464.2. Based on the distribution and redistribution patterns of ethanol after death, we can infer that the BAC of the deceased was 141.8-334.1mg/100mL when his carotid artery was cut and there was a postmortem redistribution of alcohol. Based on the EtG and EtS as the biomarker of drinking, we can determine that he drank alcohol before his death. Based on the relationship between EtG and EtS levels in blood, we can estimate that the deceased drank alcohol for 1.823 hours before the death; According to the Widmark formula (r=A/P·C) and the ethanol distribution constant of Chinese people, The amount of alcohol consumed by the deceased can be roughly estimated as 189.4~446ml(50%). Through the first case, we demonstrated the solution to the four challenges mentioned above. For example 2 and 3, there were a living perpetrator and an victim died 3 hours , whose blood were collected 3 hours after the accident, with the BAC of 17.9mg/100ml and 77mg/100ml. Based on the rate of ethanol elimination among Chinese people we can calculate the BAC at the time of the accident as 47.9mg/100ml and 102mg/100ml. So through the second and third cases, we demonstrated and solved the problem of inferring ethanol concentration in living and dead bodies at the time of the accident if the blood was collected after the accident for a while. For example 4, one perpetrator of the traffic accident had a blood BAC of 173.9mg/100ml at the time of the accident, and no EtG was detected in the blood after the blood was stored for 114 days, he was determined not drunk driving based on the EtG as the biomarker of drinking, but the vitreous humor, peripheral blood should be collected and the EtS should also be tested simultaneously. For example 5, eight days after a woman went missing, she was found dead in a well. The autopsy showed typical signs of strangulation. The ethanol content in the heart blood and stomach contents were 272mg% and 20mg%, and EtG was not detected in the blood after stored for 3 weeks. Ethanol in the heart blood was determined to be produced after the death, but external blood and vitreous fluid should be still collected, and ethanol, EtG, and EtS were tested simultaneously to determine the source of ethanol in the heart blood. For example 6, one woman was killed by covering her mouth and infused with 1000 ml (50%,V/V)wine after death. Autopsy revealed symptoms of strangle, the heart blood alcohol content was detected as 1680mg/100ml. It is believed that this woman suffered from strangle, however, more body fluid and body tissues should be collected for detection of alcohol, EtG and EtS to determine whether ethanol entered the body before or after the death. Based on the above six typical cases, a virtual simulation experimental system, named as the post mortem redistribution of ethanol and its application(http://www.ilab-x.com/login) ,had been developed for training freely.

Conclusion: Based on the study on the forensic toxicokinetics of alcohol in Chinese, we can answer the five challenge in alcohol rated case Identification in China, which are to estimate the alcohol concentration in living body or corpse, distinguish the postmortem production of alcohol and antemortem taken alcohol, identify antemortem taken alcohol and postmortem given alcohol and determine the time, dose of drinking for Chinese.

Fatal insulin overdoses: A case report and analysis of insulin in postmortem samples

PM P–29

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Background & Aims: Due to the rapid degradation of insulin and its analogues in the human body, the pathological damage caused by poisoning is not specific, the difficulty of injection is small, there are few marks on the body surface, and it is convenient to obtain. Therefore, the cases of suicide or homicide by overdosing on insulin injections

are gradually increasing. In 1994, Chi Tong reported the first case of homicide by insulin in China. Later, there were also cases of doctor Wei killing his wife with insulin in 2007, and nurse Tao killing her boyfriend with insulin in 2015. These cases have also attracted more and more attention.

Insulin aspart replaces the 28th proline on the human insulin beta chain with aspartic acid, which exists as a monomer and can be directly absorbed by the human body. It belongs to the fast-acting insulin analogues. Following an investigation, a deceased individual was discovered at his residence along with boxes of insulin aspart and used insulin pens. Forensic experts need to determine the cause of death of this man and provide objective evidence for the case.

Methods: We performed quantitative and qualitative analysis of insulin aspart in blood, urine, vitreous humor, and skin tissue suspected to have been injected with insulin using ultrafiltration method combined with LC-MS/MS. We took 0.1 mL (g) of the samples respectively, added 400 µL of 10% acetic acid aqueous solution containing 0.1% plasma, transferred it to a 100KDa ultrafiltration tube, oscillated for 10 minutes, centrifuged at 14000 rpm for 10 minutes, and then took the filtrate for analysis (Phenomenex Biozen Peptide XB-C18 column, SCIEX Triple Quad™ 6500+).

Results & Discussion: The contents of insulin aspart were 2.99 µg/mL, 10.5 ng/mL and 20.5 ng/g respectively, in urine, vitreous fluid, and skin tissue suspected of being injected with insulin of the deceased male. We confirmed the deceased was injected with a large amount of insulin aspart before death. The method we have established is accurate, effective, rapid, and simple, which can meet the need of insulin aspart detection and serve as a valuable reference for identifying other types of insulin substances.

Conclusion: The blood of the male deceased caused the degradation of insulin aspart due to hemolysis, which is difficult to test. However, it is crucial to focus on determining the insulin levels in alternative body fluids such as vitreous humor, bile, urine, and adjacent skin tissues at the injection site, the content can also provide data support for forensic toxicology analysis.

PM P-30

Postmortem alcohol exposure leads to generation of EtG and EtS in Bama minipigs and rhesus macaque

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Background & Aims: Ethyl glucuronide (EtG) and ethyl sulfate (EtS) have been used as biomarkers to distinguish between postmortem and antemortem alcohol consumption, especially EtG and EtS in vitreous humor. Some studies showed EtG was detected in ethanol spiked human blood stored at 37°C for 24 hours. However, it is currently unclear whether EtG and EtS could be generated in corpses which have undergone postmortem alcohol gavage at high ambient temperature. Based on a previous study conducted by our research group, EtG and EtS were detected in the cardiac blood, peripheral blood, liver, and lungs of rabbits one hour after death following intragastric administration of ethanol. To further verify that postmortem alcohol exposure leads to the generation of EtG and EtS in corpses, we investigated this hypothesis using models of postmortem alcohol gavage in Bama minipigs and rhesus macaques, observing the generation of EtG and EtS in both species.

Methods: Twelve male Bama minipigs and four male rhesus macaques killed by excessing carbon dioxide inhalation. The twelve male Bama minipigs were divided into two groups (n=6) and stored at 20°C and 37°C, respectively. Four male rhesus macaques stored at 37°C. One hour after death, three pigs in each group were given China liquor (14mL/Kg, 60% vol.) by gavage. The other three pigs were given alcohol 2 hours after death. Three rhesus macaques were given China liquor (12.6mL/Kg, 60% vol.) by gavage, another one was given normal saline by gavage. After 24 hours, the concentration of ethanol, EtG and EtS were detected in cardiac blood, peripheral blood, vitreous humor, urine, liver, lung, kidney, spleen, brain, lower limbs muscle and testis. In addition, one rhesus macaque was given same dose China liquor by gavage to observe the metabolism process of EtG and EtS.

Results & Discussion: For Bama minipig, in two groups, the concentration of ethanol in tissues (0.02-25.19 mg/g) was higher than that in body fluids (0.02-1.47mg/mL). Moreover, EtG and EtS were not detected in tissues. At 20°C, only EtG was detected in cardiac blood, peripheral blood and vitreous humor, whereas at 37°C, both EtG and EtS were detected in some individual.

For rhesus macaque, the concentrations of ethanol in brain (0.06mg/g), lower limbs muscle (0.11mg/g) and testis (0.09mg/g) was lower than that in other tissues (0.67-34.17mg/g). Except for urine, brain, lower limbs muscle and testis, EtG and EtS were detected in tissues and body fluids in rhesus macaque following intragastric administration of China liquor at one hour after death. And the concentrations of EtG were not significantly different from those of EtS in liver, lung, kidney, spleen. It is noteworthy that the concentrations of EtG were approximately one

orders of magnitude higher than EtS in the rhesus macaque given China liquor by antemortem gavage. To be more specific, the concentrations of EtG were 0.21–87.13 μg/mL whereas EtS were 0.04–2.83 μg/mL.

EtG and EtS are characterized by the net addition of glucuronic acid and sulpho to ethanol. This clearance pathway is catalysed by the UDP-glucuronosyltransferases (UGTs) and sulfotransferases (SULTs) superfamily of enzymes. Moreover, UGTs and SULTs are predominantly expressed in liver, lung, kidney and small intestine. The presence of EtG and EtS in corpses that have undergone postmortem alcohol gavage may be attributed to the enzymes in the body not being immediately inactivated after death. EtG and EtS were generated in tissues such as the liver, lungs, and kidneys, and then diffused into the cardiac blood, peripheral blood, and vitreous humor.

Conclusion: It is risky to detect EtG and EtS in only one tissue or body fluid, especially in cardiac blood, peripheral blood or vitreous humor. Therefore, when utilizing EtG and EtS as biomarkers to distinguish between postmortem and antemortem alcohol consumption, urine, testes, and limb muscles would serve as robust materials for analysis. Additionally, the concentration ratio of EtG to EtS in the same tissue could further aid in distinguishing between postmortem and antemortem alcohol consumption. This study was supported by two grants from National Natural Science Foundation of China (No. 82130056 and 82072116 to Keming Yun) and Shanxi Province Key Research and Development Program (No. 202302130501007 to Keming Yun)

Investigating post-mortem redistribution of drugs in a cohort of suspected unnatural deaths in Cape Town South Africa

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Background & Aims: The interpretation of post-mortem toxicological analytical results is complex, specifically, due to the phenomenon of post-mortem redistribution (PMR). The latter needs to be taken into consideration when determining if, and to what extent a drug contributed to death. Additionally, case specific features also play a role and therefore no specific drug concentration can be deemed 'fatal'. By contributing data to the larger knowledge pool, we can better understand how different drugs behave in the post-mortem setting, thereby assisting toxicologists and pathologists to come to a rational conclusion regarding the post-mortem toxicological results on a case-by-case basis.

Methods: This study is the first of its kind in South Africa, aiming to investigate the extent of PMR of common drugs of use and misuse. To this end, paired admission femoral blood and autopsy femoral and cardiac blood samples were tested on a quantitative LC-MS/MS panel for of 31 commonly misused drugs. Where enough cases were present per drug, C/P ratios were calculated and compared to literature.

Results & Discussion: C/P ratios for acetaminophen, amphetamine, methamphetamine and nor-carboxy-tetrahydrocannabinol in the current study are similar to previous studies with the exception of diphenhydramine, which had a much lower C/P ratio. The latter may be as a result of the current study's small data set, long PMI intervals or due to drug-drug-interactions as in combination, methamphetamine, methaqualone and diphenhydramine are known as 'Mandrax' - a drug commonly abused in the Cape Metropole.

Significant pairwise differences were found for amphetamine, methamphetamine and nor-carboxy-tetrahydrocannabinol. These concentration differences were found between autopsy femoral (F2) and cardiac (C) concentrations, highlighting the possible pitfall for overinterpreting findings if only cardiac blood were to be analysed. Amphetamine and nor-carboxy-tetrahydrocannabinol had no significant difference when comparing admission femoral (F1) and autopsy femoral (F2) concentrations, supporting the recommendations for choosing peripheral blood samples over central sources when collecting specimens for toxicological analyses at the time of autopsy, as this is likely to be a more accurate representation of the concentrations at the time of death. There was also a significant difference in concentration when comparing results from autopsy femoral blood (F2) and autopsy cardiac blood (C), even though these are usually collected within a short timespan from one another, once again emphasising that central blood samples tend to reveal higher drug concentrations. The latter may be as a result of diffusion from nearby drug reservoirs, such as the stomach and liver and/or concentration of drugs within the heart tissue.

It is interesting to note that methaqualone (n =33) revealed no significant concentration differences between the different time points, suggesting that the drug is more stable in the post-mortem period.

C/P ratios for acetaminophen, amphetamine, diphenhydramine, methamphetamine, methaqualone and nor-carboxy-tetrahydrocannabinol were above 1, suggesting that they undergo PMR to some extent. Acetaminophen, methaqualone and nor-carboxy-tetrahydrocannabinol had C/P ratios close to one, which may indicate that these drugs undergo PMR to a lesser extent than the other drugs.

Amphetamine (n = 13 ; 11.9 %) is the only drug in this study which showed significant concentration differences between F1 and F2 when PMI is considered, supporting the recommendations that samples must be collected as soon as possible after death.

A total of 109 suspected unnatural cases admitted to the mortuary were included, of which 61 (56%) yielded positive toxicology results. Cardiac/peripheral (C/P) ratios were calculated and significant pairwise differences with Bonferroni correction were found for amphetamine, methamphetamine and nor-carboxy-tetrahydrocannabinol, corresponding with current literature. Additionally, where the literature was previously lacking data on the PMR of methaqualone, this study suggested that it is less likely to undergo PMR.

Conclusion: To the best of our knowledge, this is the first study of its kind in South Africa. The results of 109 authentic autopsy cases in a practical mortuary setting were analysed for the 31 most commonly abused drugs in the mortuary's drainage area. This included cases with short and long post-mortem intervals. This study adds valuable data to the existing research with regards to the propensity, extent and potential of post-mortem redistribution of acetaminophen, nor-carboxy-tetrahydrocannabinol, amphetamine, methaqualone and methamphetamine. It gives a more current insight into the burden of illicit drug use in the setting of deaths in the West Metropole.

While there is currently no single solution to eliminate all the factors causing these difficulties, there are strategies to overcome some of the problems when facing PMR. A multipronged and individualised approach should be followed throughout the entire post-mortem process for each case. Starting at minimizing the PMI, continuing through to appropriate sampling technique and storage, sample preparation and analysis, result reporting and possible alternative strategies.

PM P–32

Qualitative post-mortem toxicological urine screening experience over 18 months for determining cause of death

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Background & Aims: The damage linked to drug use considerably damages the person using drugs and often leads to death. Reducing these premature and avoidable deaths is a major challenge for health policy. introduced by thisThis study aims to assess the prevalence of drugs of abuse detected in decedents using urine immuno-chromatographic testing, between January 2022 and june 2023.

Methods: A sample of 128 cases from autopsy records at Mustapha University Hospital's Forensic Medicine Department was reviewed over a 20-month period. Psychoactive substances were detected using rapid immuno-chromatographic urine testing of 12 drugs (pregabalin, tramadol, burpenorphine, opiates, tricyclic antidepressants, benzodiazepines, barbiturates, amphetamine, metamphetamine, MDMA, cocaine, and THC).

Results & Discussion: The results show that pregabalin was identified as the most frequently detected psychoactive substance (24% of cases). followed by cannabinoids (23% of cases), then opioids (20.8% of cases), and cocaine and amphetamines were positive in just 6.5% of cases. Furthermore, 70% of positive cases are polydrug users, whose most observed association is that of pegegabalin with opioids (heroin, buprenorphine, or tramadol).

These qualitative results underline a significant prevalence of pregabalin among the psychoactive substances detected post-mortem and the probable crisis of opioid consumption in Algeria. In addition, the majority of drug addicts consume several types of drugs.

Conclusion: The need to better understand the prevalence of post-mortem-detected drugs and concurrent use of multiple substances is important to guide public health policies aimed at reducing preventable premature deaths related to drug use.

PM P–33

Beyond the grave: Revealing the sinister truth of para-phenylenediamine poisoning through exhumed specimens

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Background & Aims: Revelation of unnoticed instances of poisoning prior to the burial by toxicological analysis is of significant forensic importance.

A request for toxicology analysis of evidence samples from a case of suspicious death was submitted to author's laboratory. A wife poisoned her husband, but this fact remained unknown to everyone until after his burial. Three months later, prompted by the victim's brother and after the confession of the victim's wife, exhumation was conducted to unveil the cause behind death. Putrefied specimens from the liver and stomach were submitted with a request for identification of an unidentified poison.

The aim of this study is to contribute valuable data in the realm of forensic toxicology, with a focus on para-phenylenediamine (PPD) in exhumed specimens to assist in investigations of suspicious deaths.
PPD, locally known as "Kala Pathar" in Pakistan, is a constituent of hair dye and henna. It's easy availability and lethality makes it versatile for use as a poison. Possessing lethal characteristics, it may lead to death due to its effect on multiple organs. Clinical manifestations include rapid development of severe facial edema, hardening of the tongue, and laryngeal spasm with respiratory distress, which often require tracheostomy in phase I. Rhabdomyolysis and acute tubular necrosis supervene in phase II.

Methods: Screening of the submitted specimens was initially performed for phosphide and cyanide making use of a silver nitrate and cyanocobalamin strips, respectively. Putrefied materials were extracted using the QuEChERS technique and an Agilent EMR (C18) powder assisted clean-up step. The samples were run on a GCMS (Agilent-7890A/5975), in scan mode, using a DB-5MS, 15 m × 250 µm × 0.25 µm capillary column.

Results & Discussion: PPD and monoacetylated PPD (metabolite of PPD) were identified in putrefied material from the stomach site, while monoacetylated PPD was identified in putrefied material from the liver site on the basis of their retention time, mass spectral comparison, and library match from the "NIST 20" database.

After being ingested, PPD undergoes metabolism through both N-acetyl transferases type I and type II, resulting in the formation of monoacetylated PPD and diacetylated PPD. The first metabolite, monoacetylated PPD, is short-lived and further converted to diacetylated PPD.

Detection of PPD with monoacetylated PPD in a stomach-site sample, and detection of only monoacetylated PPD in a liver-site sample, shows recent exposure to PPD before death. Persistence of PPD and its metabolite in the specimens, even following a burial period of three months, indicates the stability of compounds, providing valuable insights for forensic toxicologists.

Conclusion: Toxicological analysis on exhumed specimens has paramount importance, as preferable biological matrices (blood, urine) are inaccessible to forensic toxicologists. Given the decomposition of specimens, which lacks valuable autopsy findings and renders histopathological assessment inconclusive, the emphasis shifts to toxicological analysis. Furthermore, despite a three-month burial period, the detection of PPD in highly putrefied exhumed specimens underlines the significance of forensic toxicology in establishing the cause of death.

Also, the findings of this study advocate for extension of the toxicology screening scope to include locally available household poisons.

The significance of acetaldehyde determination in post-mortem samples

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Background & Aims: Blood alcohol determination presents a routine analyses in forensic toxicology. In post-mortem samples other volatiles compounds (VOCs), including acetone, formic acid, methanol, 1 – and 2 – propanol, and acetaldehyde are frequently detected. Despite the ante-mortem exposure to these compounds, their presence in post-mortem samples can be result of biochemical processes occurred almost immediately after the death, due to decline of the oxygen concentration, leading to anaerobic metabolic reactions. VOCs has been widely evaluated in different post-mortem periods, under the influence of various microorganisms, under different sample's storage conditions toward defining the pathway, the amount and the period of their production in favor of their use as a markers for specific forensic purposes. In forensics, research on acetaldehyde has been focused mainly in estimation of the relationship between post-mortem alcohol production and ante-mortem alcohol consumption supporting the interpretation of post-mortem blood alcohol results. Purpose of this study was to evaluate a real post-mortem cases with positive acetaldehyde findings, testing the hypothesis for its usefulness as a marker with wider forensic significance.

PM P–34

Methods: Samples included in this study were obtained during the autopsies performed at the Institute of forensic medicine, criminology and medical deontology, Medical faculty, Ss Cyril and Methodius University – Skopje. Selection of the samples was based on undoubtedly data about the corps condition considering the existing of predefined signs and stage of putrefaction. Cases with partially or totally absent rigor mortis and without visible putrefaction were excluded from the study. In total 343 blood samples from routine casework were included in the study. Venous post-mortem blood samples were kept in a tube with NaF at +4°C until analyses (within the five days of sampling). VOCs analyses were performed using validated method for routine ethyl alcohol analyses on GC 2010 Plus (Shimadzu, Japan) with Headspace auto sampler AOC 5000, equipped with InertCap 624 (30 m x 0,53 mm x 3µm). As a positive finding for acetaldehyde presence were considered cases with concentration of acetaldehyde ≥ 0.013 g/L.

Results & Discussion: After the measurement of acetaldehyde concentration results were classified in four groups as follows: I – putrefied cases with acetaldehyde (n = 9); II – putrefied cases without acetaldehyde (n = 7); III – not putrefied cases with acetaldehyde (n = 18); IV – not putrefied cases without acetaldehyde (n = 309). According to the distribution among cases, acetaldehyde was determined in 56% of the putrefied cases with average concentration of 0.041 g/L. These findings are in line with literature data suggesting that acetaldehyde presence can be used as a marker for putrefaction. However, concentrations measured in our study are significantly lower than those reported in other studies on the similar topic. Other interesting finding is that in the group II there are two cases with blood alcohol concentration (BAC) between 2 – 3 g/L. Analyses of the results from not putrefied cases has shown that acetaldehyde was present in 5.5% of the samples, with average concentration of 0.03 g/L. All of the cases from group III, as expected, had positive findings for ethyl alcohol due to the fact that acetaldehyde is product of ethyl alcohol metabolism whether consumed ante-mortem or post-mortem produced. In group IV 20.71% (n = 64) of the cases were positive for ethyl alcohol, without acetaldehyde presence. Taking in account presented results cannot be drawn strong evidence for acetaldehyde use as a predictive marker for post-mortem production of ethyl alcohol. In order to examine the versatility of acetaldehyde determination in post-mortem samples, few samples, with extremely high concentrations of acetaldehyde were separately evaluated. In the case number 1 BAC was 1.45 g/L and acetaldehyde concentration 0.0959 g/L. This case belongs to group I. Case number 2 with BAC 0.733 g/L and acetaldehyde concentration 0.0922 g/L, and case number 3 with BAC 0,121 g/L and acetaldehyde concentration 0.0776 g/L belong to the group III. Even though all three cases have positive BAC they were characterized with significantly higher acetaldehyde concentration compared with the other cases from appropriate groups. Cases history revealed that case number I was found in the cave, case number 2 had ischemic cardiac arrest and case number 3 was fire victim. In common all three cases has been in hypoxic state before the death, which derive a consideration that hypoxic state can lead to higher production of acetaldehyde.

Conclusion: Acetaldehyde presence in post-mortem blood samples can suggest to a putrefaction changes, however, its concentration cannot be decisive prediction for ethyl alcohol use because its presence is found in cases with ante-mortem used and post-mortem produced ethyl alcohol. Presence of high acetaldehyde concentration in the post-mortem samples can be result of a preexisting hypoxic state before the death, however, it is a hypothesis which requires additional examination of the mechanism of its occurrence trough well established scientific studies on a huge number of samples.

PM P-35

The significance of adding cyanide testing to the standard toxicological analyses panel for poisoning cases

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Background & Aims: Cyanide is one of the most notorious and rapidly acting poisons, from spy novels to murder mysteries. Different routes of intake include inhalation of hydrogen cyanide gas, skin absorption, or ingestion of cyanide salts. Cyanide inhibits electron transport, thereby majorly affecting brain and cardiac functions. Cyanide can be found in many different forms, sodium cyanide (NaCN), potassium cyanide (KCN), hydrogen cyanide (HCN), cyanogen chloride (CNCl), and calcium cyanide (CaCN). Cyanide naturally occurs in almonds, lima beans, soy, and spinach. Cyanide is even a byproduct of metabolism in the human body. Cyanide salts are used in the electropolishing of gold jewelry, and in the manufacture of multiple other chemicals. The aim of this study is to highlight the importance of circumstantial information and the inclusion of cyanide testing in routine toxicological analysis panels for poisoning cases.

Case History:
The current case study is about a 40-years-old goldsmith who committed suicide by using potassium cyanide, locally known as "andaa potash." The deceased had grave marital problems and he was also consuming tranquilizers. Reportedly, shortly after ingestion, he experienced poisoning symptoms, including throat sounds and loss of consciousness. Despite prompt medical attention, he succumbed to the poison before reaching the hospital. Blood was oozing out of his mouth, and he was having difficulty breathing, indicating severe respiratory depression.

Methods: Blood and stomach contents were submitted to the author`s laboratory for toxicological analysis. The submitted specimens were screened for the presence of phosphides and cyanide using a silver nitrate and a cyanocobalamin strips, respectively.

Confirmation and quantitation of cyanide was made using 2 ml of each sample (sample, calibrators and controls) placed into a 20-ml headspace vial. 100 µL of glacial acetic acid was added to the sample vial, and heated for 30 min at 70 °C in an oven. One ml of the headspace sample was removed from a sealed sample vial, and manually injected into a gas chromatograph coupled with a nitrogen phosphorous detector (GC-NPD) using HP-PLOT/Q at 270 °C, 30 m × 530 µm × 40 µm. The oven programming of the method starts with an initial temperature of 50 °C for 1 minute, ramped to 150 °C at a rate of 50 °C/min, and is held for 2 minutes (total run time: 5 minutes).

Hydrogen cyanide was identified by its characteristic retention time with respect to calibrators retention time and the positive control sample. Six calibrators containing cyanide concentrations of 0.2, 0.5, 1, 2, 5, and 10 mg/L were concurrently analyzed.

Results & Discussion: Initial colorimetric screening indicated the presence of cyanide in all the specimens. Cyanide was identified and quantitated in the blood and stomach contents, based on retention time on a GC-NPD.1348 mg/L of cyanide was found in blood sample, which is significantly higher than the reported lethal concentration (1–100 mg/L) of cyanide. 4583 mg/L cyanide was found in the stomach contents. Quantitated concentrations of cyanide in blood and stomach contents, along with autopsy findings and circumstantial evidence, clearly indicate cyanide poisoning as the cause of the victim's death.

Conclusion: This study underscores the importance of targeting cyanide during routine toxicological analysis. Cyanide, a deadly poison, is still in use in the modern world. Cyanide is not targeted during routine toxicological analysis by most of the forensic toxicology laboratories across the world and it may go undetected in cases with unclear history. Cyanide analysis panels must be included in routine toxicology analysis, especially in poisoning cases.

Development and validation of GC-MS method for screening and quantitation of chloroform in blood using liquid-liquid extraction

PM P-36

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Background & Aims: Chloroform is extensively used in the past as an anesthetizing agent and an additive in certain pharmaceutical preparations. Its use on humans or animals has been banned because of its carcinogenic properties. Its use as synthesis intermediate and extraction solvent in laboratories and industries, still prevailing. Although long superseded as an analgesic and anesthetic agent in clinical practice, chloroform is still encountered occasionally in suicidal, homicidal and drug facilitated sexual assault (DFSA) cases in clinical and forensic toxicology setups. Hence knowledge of analytical and clinical toxicology analysis of chloroform in biological matrices is very critical in establishing the cause of death by determining its lethal or toxic range. Extensive review of literature unveiled specialized techniques usage for chloroform detection, such as Solid Phase Micro Extraction (SPME) and the use of specific GC columns tailored for volatile analysis.

Over the years, different procedures have been published for the qualitative and quantitative determination of chloroform but none of the method uses liquid-liquid extraction of chloroform. This study aimed to develop and validate a method for the qualitative and quantitative analysis of chloroform with liquid-liquid extraction using Gas Chromatography-Mass Spectrometry (GC-MS), in shorter possible run time than previously published procedures, capitalizing on the available resources.

Case History:
A postmortem case involving a 38-year-old female victim was presented, wherein the author's laboratory was tasked with analyzing suspected poisoning. As per the provided history, the female was subjected to torture and beaten to death due to a property dispute with her husband. Postmortem examination revealed bruises on various body parts and discoloration around the lips and nose. Petechial hemorrhages are present on inner side of lips and eyes. Petechial hemorrhages and congestion was present in both lungs. Heart, liver, spleen and kidney were congested. There was also bleeding from nose. Initial forensic analyses for local poisons, different classes of drugs and pesticides yielded negative results. Subsequently, crime scene samples, including a handkerchief and a bottle labeled as chloroform, were submitted for analysis. Accordingly, chloroform analysis was conducted on postmortem samples to assess the potential ingestion.

Methods: A method for quantitation of chloroform on GC-MS using liquid-liquid extraction, was developed and validated. Dichloromethane served as the internal standard (I.S.). The separation was accomplished on a GC-MS system (Agilent-7890A/5975) equipped with a capillary column (DB-5 MS, 15m × 250µm × 0.25 µm) using helium

as the carrier gas. Chlorobutane was used as an extraction solvent. Quantitation of chloroform was performed in Selected Ion Monitoring (SIM) mode, with a remarkably short runtime of 1.5 minutes. Retention times of I.S. and chloroform were 0.5 min and 0.7 min respectively. Selected ions for I.S. was 84 and 88 while for chloroform was 83, 85 and 82. 1 µL of sample was injected with a split ratio of 50:1 in split mode.

Results & Discussion: The validation study was conducted in accordance with OSAC (Organization of Scientific Area Committees for Forensic Science) guidelines, with calibrators, and controls run in triplicate over a period of six days. Quantitation was performed using a multi-point calibration based on the peak area ratio between analyte and I.S. using linear calibration model. The method exhibited excellent linearity (r2 > 0.990) over the concentration range of 0.150 to 10 mg/L, with no observed carryover beyond the highest concentration level (10 mg/L) tested. The Limit of Detection (LOD) and Limit of Quantitation (LOQ) for chloroform were determined to be 0.150 mg/L and 0.310 mg/L, respectively. Intraday precision (CV < 5.25%), interday precision (CV < 4.59%), and accuracy (± 14.35%) were within the accepted criteria for analytical methods employed in forensic toxicology. The result of interference study revealed no interference at relevant retention times and with selected ions. Furthermore, processed sample stability testing demonstrated stability for 72 hours on the GC-MS Auto-sampler. The validated method was successfully applied to screen and quantitatively analyze chloroform in real forensic case samples, revealing chloroform concentration falling within the fatal range in blood i.e 7.2 mg/L. Chloroform was also detected in stomach contents, handkerchief and liquid bottle collected from the crime scene.

Conclusion: Our proposed method holds promise as a cost-effective alternative for laboratories because it does not require dedicated instrument and column. Its shorter run time can save expensive carrier gas as well as time. Furthermore, to the best of our knowledge, no method for chloroform detection and quantitation using liquid-liquid extraction by GC-MS, has been reported. Our presented method is sufficiently rapid, simple, economical and sensitive that complies with OSAC validation guidelines. Application of this method reveals cause of death in real forensic case by establishing fatal levels of chloroform in blood, along with detection of chloroform in stomach contents and crime scene samples.

PM P–37

Propranolol fatalities; an increasing trend in cases from our post mortem workload across England and Wales, UK

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Background & Aims: Propranolol is a beta-adrenoceptor blocker, which may be prescribed for the management of hypertension, angina, cardiac arrhythmias and anxiety. Propranolol has a half-life of 2-4 hours and a volume of distribution of 3-5 L/kg. Propranolol toxicity has been associated with significant side effects including seizure, hypoglycaemia, cardiovascular collapse, respiratory depression, coma and death. Propranolol may interact with other central nervous system depressants enhancing toxicity. According to the 2022/2023 annual report published by the National Poisons Information Service (NPIS), 318 patients intentionally overdosed on propranolol in the United Kingdom; with 12 of these being fatal. An increase in cases involving excess propranolol ingestion has also been observed by the Toxicology Unit, University Hospitals of Leicester (UHL) NHS Trust, over the previous two years. Our post-mortem toxicology service analyses samples from approximately 25% of HM Coroners across England and Wales, receiving around 4500 cases per year from a number of jurisdictions. Data from our laboratory was searched; any cases in which propranolol use was deemed to have caused or contributed to death were reviewed. Post mortem blood propranolol concentrations, any additional drugs / medications detected and demographics of the deceased were collated.

Methods: Cases in which propranolol was detected were reviewed from January 2022 to February 2024 inclusive. Initial toxicological screening was performed using high resolution accurate mass-mass spectrometry (Waters Xevo G2-XS Quadrupole Time-of-Flight mass spectrometer). This analysis was performed primarily in post mortem plain femoral blood (other sample types included admission serum or admission EDTA blood, plain blood (unspecified site), preserved blood (femoral and unspecified sites) and, in a small number of cases, sub-clavian blood). For cases in which propranolol was detected on screening, quantitative analysis of propranolol (and all other drugs detected which may have caused / contributed to death) was performed by liquid chromatography-tandem mass spectrometry (LC-MS/MS; Agilent 6410 triple quadrupole) for the majority of analytes. All post mortem cases were also subjected to ethanol analysis by head-space gas-chromatography with flame ionisation detection (HS-GC-FID; Agilent 7697A headspace sampler and 6890 GC-FID) to determine whether ethanol was present and, if so, at what concentration.

Results & Discussion: Propranolol was detected in 294 out of a total of 10,219 cases (2.9 %) over the 2 year and 2 month period reviewed between January 2022 and February 2024. Propranolol was detected in combination with other drugs in the majority of cases. Of the 294 cases, 43 (14.6%) had post mortem blood propranolol concen-

trations at a potentially lethal level of greater than 4,000 ng/mL. The mean propranolol concentration was 13,861 ng/mL (range, min-max, 4,348 ng/mL to 88,126 ng/mL), median (interquartile range) 9,229 ng/mL (6,184-16,163 ng/mL). Mean age was 43 years (range, min-max, 18-80 years) with a preponderance of female individuals (73 %; n=32). In the majority of cases (67%, n=29) individuals were found unresponsive or deceased at home. In many cases (33%, n=14) empty blister packets of medication were located at the scene. Of those in which a medical history was provided, the majority (71%, 24 of 34 cases) had a history of depression. Anti-depressant medications, including selective serotonin reuptake inhibitors (SSRIs) were detected in 77% of cases (n=33), either alone or in combination; citalopram (10), mirtazapine (9), venlafaxine (7), fluoxetine (6), sertraline (4) and paroxetine (1). There were 7 cases in which cocaine and/or its metabolites were detected; of interest, the majority of these cases (n=4) fell within the first two months of 2024. Of the total number of cases reviewed (n=294), the percentage at which propranolol was detected at a potentially lethal concentration were 6.6%, 17.6% and 32%, for 2022, 2023 and 2024 respectively (with 2024 based on data for the first two months of 2024 alone). These findings correlate with an increase in propranolol related fatalities in our case workload from less than one case per month in 2022 to five cases per month in the first two months of 2024.

Conclusion: We report an increase in the number of deaths associated with excess propranolol ingestion, from toxicological analyses alone, in line with findings from the NPIS annual report (2022/2023). In many cases propranolol was detected in combination with anti-depressant medication which may exacerbate the cardio-toxic effects of propranolol. Due to the increased prevalence of propranolol-related fatalities, our case series also highlights the importance of the detection and subsequent quantitation of propranolol across all age ranges. Our most recent data also suggests an increase in cocaine use in combination with propranolol which is likely to enhance adverse cardiac effects.

Suicide by self-administration of pethidine, propofol and anxiolytics : a case report

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Background & Aims: Recreational use of anesthetic and sedative agents in health care practitioners is becoming an increasing problem due to its easy availability and, therefore, they become vulnerable to misusing or abusing of these drugs. The authors present a fatal case involving a a 50-year-old man, nurse, found dead in a hospital room, with intravenous access devices in the forearm that were attached to two packages of saline solutions. Some empty packages were close to the victim. A white viscous fluid found at the death scene was send to analysis. The expert's investigation at the scene indicated possible propofol poisoning.

Methods: The blood samples were initially subjected to a qualitative analysis. A systematic toxicological drug screening was carried out in blood with a combination of enzyme immunoassays (ELISA) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. Femoral blood alcohol concentration was measured by gas chromatography with headspace (GC/FID) technique. Due to suspected propofol intoxication, a specific analytical methodology was developed in order to detect and quantify this substance in the postmortem samples.

Chromatographic analysis of propofol was preceded by protein precipitation of 0.1 mL of blood samples with acetonitrile. Chromatographic separation was achieved with a reversed-phase column, using a gradient elution of 2 mM aqueous ammonium formate (0.1% formic acid), and 2mM ammonium fluoride in methanol, at a flow rate of 0.4 mL/min, with a run time of 10 minutes.

Results & Discussion: Toxicological results revealed the presence of the anesthetic (propofol) in femoral blood (33.5 mg/L) and heart blood (113.3 mg/L), and also in the white viscous fluid found at the death scene. The remaining laboratory analyses revealed the presence of nordiazepam, temazepam and metoclopramide in therapeutic concentrations, but toxic blood concentrations of midazolam (1473 µg/L), diazepam (5182 µg/L) and pethidine (8622 µg/L). The blood alcohol analysis result was positive (0.18 g/L) as well as positive for opiates (morfine < 25 µg/L). Pesticides were not present in the analysed samples.

Conclusion: The development of the method for confirmation and quantification of the anesthetic propofol was crucial given the suspicion of poisoning by this substance. However, laboratory analysis has also revealed the simultaneous presence of an opioid analgesic agent (pethidine) and anxiolytics in high blood concentrations. These findings have allowed to identify a case where several drugs were abused and whose synergy of effects and intoxication, that were not considered at first, were lately found to have contributed to the medico-legal etiology of suicide.

PM P–39

Comprehensive toxicological analysis in a case of unexpected fatal codeine intoxication

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Background & Aims: Codeine, a commonly prescribed opioid analgesic and antitussive, is widely used either alone or in combination with other medications. This substance is typically administered orally and is well absorbed from the gastrointestinal tract. After being absorbed, it is distributed throughout the body and crosses the blood-brain barrier to reach the central nervous system, where it exerts its pharmacological effects. Metabolized extensively by the liver enzyme CYP2D6, codeine is converted to its active metabolite, morphine, along with other metabolites such as norcodeine, codeine-6-glucuronide, and morphine-3-glucuronide. Despite its therapeutic benefits, codeine can induce adverse effects such as drowsiness, constipation, nausea, and respiratory depression, particularly at higher doses or in susceptible individuals.

We present an unusual case concerning a 60-year-old woman that was found dead at home by his husband, when he came back home for lunch. In that morning, he left her sleeping in the bed. She had diarrhoea in the previous 48h, was obese, and suffer of asthma and psychiatric illness. Her usual prescribed medications included the antidepressants escitalopram and triticum, the anticonvulsant oxcarbazepine (used primarily to treat epilepsy and bipolar disorder), the anxiolytic lorazepam, the antipsychotic quetiapine, the asthma control montelukast and the atorvastatin used to treat hypercholesterolemia. There was no history of ingestion of any formulation containing codeine. Autopsy showed no evidence of trauma. A slight cardiac hypertrophy related with a hypertensive cardiopathy was observed macro and microscopically. Fragments of white pills were found in stomach and a dark liquid content until the jejune.

Methods: Validated analytical procedures, routinely used in our casework, were applied to analyze peripheral and cardiac blood, urine, gastric content, and liver specimens. Initially, drugs of abuse and benzodiazepines were screened using immunoassay methodology, while ethanol screening was conducted by gas chromatography-flame ionization detector with headspace injection (GC-FID-HS). Subsequently, systematic toxicological analyses were performed using gas chromatography-mass spectrometry (GC-MS) for drugs of abuse and unknown substances, and ultra-high-performance liquid chromatography-mass spectrometry (UPLC-MS-MS) for prescription substances.

Results & Discussion: No ethanol or drugs of abuse were detected in blood samples. However, codeine was confirmed, in all analyzed specimens, in significantly high concentrations: peripheral (5,3 µg/mL) and cardiac (7,5 µg/mL) blood, suggesting recent consumption; liver (liver (9,9 µg/mL), confirming extensive metabolism; urine (206 µg/mL), indicating substantial drug excretion before death; and gastric content (197 µg/mL), confirming ingestion prior to death. Additionally, morphine, a codeine metabolite, was present in peripheral and cardiac blood at concentrations of 190 and 616 ng/mL, respectively. Furthermore, therapeutic levels of various prescription medications were detected in peripheral blood, including atorvastatin, citalopram, oxcarbazepine, paracetamol, quetiapine, norquetiapine, trazodone and its metabolite mCPP, lorazepam and its metabolite desalkylflurazepam.

Conclusion: The autopsy was negative except for a hypertensive cardiopathy, that was not severe enough to justify the death. On the other hand, toxicological results showed unexpectedly codeine in the blood, stomach, liver, and urine, in high concentrations. The pills fragments found in the gastric content reinforce its ingestion before death. Taking into account all these facts, the cause of death was attributed to codeine ingestion, although in an unknown formulation. The manner of death remains unascertained, as suicide could be a possibility according to her psychiatric antecedents, as well as an accident, if codeine had been taken to treat the diarrhoea she had.

PM P–40

Analysis of cytochrome P450 and phenoconversion in psychotropic-related deaths

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Background & Aims: In forensic toxicology, the molecular autopsy is a crucial technique. Phenoconversion (PC) is the difference between the phenotype that results from genotyping and the actual phenotype following phenoconversion. Still, for best practice, a number of factors need to be considered, including administrated drugs and diseases. These factors may lead to PC. The aim of this study was to evaluate the PC of cytochrome P450 in postmortem cases where drugs that are inhibitors of these enzymes were detected. It also aimed to assess PC's impact on the drug's metabolic ratio (MR).

Methods: Blood samples from 45 cases of psychotropic-related deaths were examined to find and measure drug and metabolite concentrations. Additionally, the HaloPlex Target Enrichment System was used to genotype each sample to be able to calculate MR (parent drug/metabolites). An activity score (AS) was assigned in order to evaluate the phenotype ofCYP2D6 (PM : poor metabolizers, IM : intermediate metabolizers, NM : normal metabolizers , and UM : ultrarapid metabolizers) based on its genotype. To differentiate the phenotypic categories resulting from

PC from those based on the real genotype (g-phenotype), the term "p-phenotype" was employed. For CYP2D6, the starting ASs of different g-phenotypes were assessed as gPM (0), gIM (0 < x < 1.25), gNM (1.25 ≤ x ≤ 2.25), and gUM (>2.25). In the case of strong inhibitor administration, the AS was multiplied by 0. Thus, any adjusted AS will be 0 and the p-phenotype will be pPM. In the case of a moderate or weak inhibitor, the AS was multiplied by 0.5. Thus, the adjusted AS will be reduced to half. For example, the gNM case (AS = 1.25 ≤ x ≤ 2.25) will be pIM (adjusted AS at 0 < x < 1.25) with moderate inhibitor administration.

Results & Discussion: The current study's findings showed a statistically significant PC rate (the cases with a change from one phenotype to another; due to exposure to medication or having disease, in comparison to cases without change in their phenotypes due to unexposure or not having disease) for the investigating cytochrome P450, along with a higher incidence of poor metabolizers (higher incidence of pPM versus gPM among the same cases) following PC. The findings of the genomic examination conducted following PC showed consistency with the MRs that were found.

Conclusion: This prompts us to concentrate on the factors mainly drugs and diseases causing PC. In forensic toxicology, PC is a sophisticated phenomenon that can greatly influence genotype interpretation, analysis, and reliable results. To validate these findings, additional study with a larger number of cases is required in the future.

Fatal intoxication involving 3,4-methylenedioxy-derived designer drugs

PM P–41

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Background & Aims: Belonging to the class of synthetic cathinones, 3,4-methylenedioxy-α-pyrrolidinohexanophenone (MDPHP) is a 3,4-methylenedioxy-derived designer (MDD) drug with a pyrrolidine moiety and an alkyl portion with six carbon atoms. Other MDD pyrrolidine derivatives belong to the alkyl homologous series (C3-C5) are the 3,4-methylenedioxy-α-pyrrolidinopropiophenone (MDPPP), 3,4-Methylenedioxy-α-pyrrolidinobutyrophenone (MDPBP) and 3,4-methylenedioxy-pyrovalerone (MDPV). MDPHP was notified to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) for the first time in 2014. In Italy, it is a controlled substance since the same year, firstly as analogue of cathinone and then as a single compound since 2020. Only two cases of death involving MDPHP have been published so far. In the case reported here, a 58-year-old Caucasian man with an history of chemsex practices was found dead in a waterway. During the autopsy, no evidence of natural disease or trauma was found to account for the death.

Methods: Blood (both peripheral and central), urine, bile, vitreous humor, gastric content, brain, liver, kidney, lungs, and pubic hair specimens were collected and submitted for toxicological analysis. MDPHP and MDPV were detected and quantified by GC-MS and UHPLC-MS/MS analysis using methods purposely developed. MDPPP and MDPBP were identified in the same specimens by HPLC-HRMS (not quantified yet).

Results & Discussion: The peripheral blood and urine concentrations were 354 and 1940 ng/mL for MDPHP, 0,1 and 0,8 ng/mL for MDPV, respectively. Neither other drugs of abuse nor ethanol were found in blood and urine specimens. Citalopram and 7-aminoclonazepam were also detected at the concentration of 528 and 353 ng/mL in peripheral blood, 4134 and 137 ng/mL in urine, respectively. MDD metabolites were further investigated by HPLC-HRMS. The combined circumstantial elements and toxicological results of the case revealed the occurrence of an acute multidrug intoxication produced by MDPHP and 7-aminoclonazepam in presence of MDPPP, MDPBP, MDPV and citalopram.

Conclusion: To the best of our knowledge, this is the first fatal intoxication case reported involving multiple 3,4-methylenedioxy-derived designer drugs and with MDPHP post-mortem concentrations in body tissues, fluids and body hair. This study underlined that the adoption of validated analytical methods for the detection of a large number of NPS with an appropriate sensitivity is pivotal for forensic toxicology laboratories.

A case report on the analysis of autopsy samples from barium poisoning death using ion chromatography

PM P–42

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Background & Aims: An 18-year-old woman was found dead at home by her family returning from an outing. An autopsy was performed on the deceased, and heart blood, stomach contents, clothing, a coke can, a plastic container labeled barium chloride, and gauze from vomit were submitted. Barium is a heavy divalent alkaline earth metal and is a non-essential nutrient for terrestrial animals, and is known to cause toxicity when the concentration exceeds a certain level in the body. Barium compounds such as barium chloride and barium hydroxide are used in the manufacture of ceramics, pesticides, and rodenticides. Insoluble forms such as barium sulfate are known to be almost non-toxic, whereas soluble forms such as barium chloride are known to be highly toxic. Barium poisoning occurs very rarely due to accidental contamination of food or water, ingestion for suicide, or inhalation exposure in the workplace. The toxic mechanism of barium is to bind to the sodium-potassium pump and block the potassium channel. Therefore, ingestion of soluble barium salt causes hypokalemic paralysis, respiratory and cardiac arrest, and death. In general, atomic absorption spectroscopy (AAS) and inductive coupled plasma-optical emission spectrometry (ICP-OES) have been used for barium analysis in biological and non-biological samples. Through this study, we would like to present a method for qualitative and quantitative analysis of barium in autopsy samples using ion chromatography, which is faster and simpler than AAS or ICP-OES.

Methods: Blood diluted with distilled water was filtered at 14,000 G using an Amicon ultra centrifugal filterTM (10kDa, 0.5mL), and filtrate diluted 0.1x from an initial concentration was analyzed by ion chromatography. Ion chromatography was equipped with a conductivity detector and a Metrosep C4 – 150/4.0 column, the eluent was 1.7mM nitric acid + 0.7mM dipicolinic acid, the flow rate was 0.900mL/min, and the column temperature was 60°C.

Results & Discussion: Barium was detected in samples from the scene of the accident and the vomit of the deceased, and 1.66 mg/L and 2.84 mg/L of barium was detected in the blood and stomach contents of the deceased.

Conclusion: Since barium was detected within a lethal concentration (0.37~23 mg/L) in the deceased`s blood, the main cause of death was barium poisoning. The LOD and LOQ were 0.03 and 0.11 mg/L, which suggests that barium quantitative analysis in biological samples using ion chromatography is a useful method.

PM P–43

A unique case of cocaine overdose: Interpretative challenges associated with cocaine and its metabolites in biological specimens

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Background & Aims: Cocaine has become a popular recreational drug among teenagers and young adults worldwide, due to its low price. Vasospasm, thrombus formation, rupture of cerebral aneurysms, intracranial hemorrhage, cardiac ischemia, stroke, seizures, and sudden death are the consequences of cocaine mediated stimulant toxicity. The reported elimination half-life of cocaine ranges from 40 to 90 minutes in humans. While a small proportion of cocaine is excreted unchanged into urine, the majority is hydrolyzed by plasma and liver carboxylesterases, yielding benzoylecgonine (BE) and ecgonine methylester (EME). Pyrolysis of cocaine during smoking results in the formation of methylecgonidine.

The interpretation of cocaine and its metabolites in postmortem specimens is a complex subject. In this case study quantitative analysis of cocaine in blood, urine, vitreous humor, bile and stomach contents was performed. Relative distribution of total cocaine (cocaine and metabolites) between blood and other postmortem samples was also evaluated.

Case History:

A postmortem case involving a 25-year-old male was presented with the history of addiction and requested to perform alcohol analysis. Attendant of deceased claimed it to be custodial torture death. There were no signs of violence on the body and autopsy findings were negative.

Analyst's laboratory performed drug and alcohol analysis on blood sample. It resulted negative for alcohol consumption but cocaine and its metabolites were detected in blood, bile, urine, vitreous humor and stomach contents.

Methods: Quantitation of cocaine and its metabolites was performed using already developed and validated method on GC-MS. Solid phase extraction (SPE) column was used for the extraction of cocaine and its metabolites along with Nalorphine as an internal standard (I.S.). The separation was accomplished on a GC-MS system (Agilent-7890A/5975) equipped with a capillary column
(DB-5 MS, 30 m × 250 µm × 0.25 µm) using helium as the carrier gas. 1 mL sample is extracted and dried at 40 °C followed by derivatization with BSTFA (N,O-Bis(trimethylsilyl) trifluoroacetamide) and 2 µL of extracted sample was injected into GC-MS. Quantitation of cocaine and its metabolites was performed in selected ion monitoring (SIM) mode, with a total runtime of 25 minutes. Retention times of EME, Cocaine, BE, I.S. are 9.7 min, 16.09 min, 16.54 min and 18.38 min respectively. Selected ions for I.S. was (455.4, 414.4), Cocaine (82.2, 105.2, 182.2, 303.3), BE (240.3, 82.2, 361.2, 105.2), and for EME was (82.2, 83.2, 96.2).

Results & Discussion: The limit of detection (LOD) and limit of quantitation (LOQ) for EME is 100 µg/L. The LOD and LOQ for cocaine and BE is 20 µg/L Calibration range for EME is 100 µg/L to 2000 µg/L. While calibration range for cocaine and BE are from 20 µg/L to 1000 µg/L. The linearity for EME (r2 > 0.994), cocaine (r2 > 0.986) and for BE (r2 > 0.999).

Quantitation of cocaine and its metabolites was conducted in blood, urine, bile, vitreous humor and stomach contents. Analysis showed concentration of EME 34.25 mg/L, Cocaine 236 mg/L and BE 30.24 mg/L in blood. Concentrations of EME 186.6 mg/L, Cocaine 120.4 mg/L, BE 94.6 mg/L in urine. Concentrations of EME 3.0 mg/L, Cocaine 70.6 mg/L, BE 10.6 mg/L in bile. Concentrations of EME 7.8 mg/L, Cocaine 2.45 mg/L, BE 1.76 mg/L in vitreous humor. Concentrations of EME 2.8 mg/L, Cocaine 146.6 mg/L, BE 7.4 mg/L in stomach contents. All of these concentrations fall within the lethal ranges for EME, cocaine and BE. Lethal range of cocaine in blood (0.1-330 mg/L), urine (0.05-402 mg/L), bile (2-468 mg/L) and vitreous humor is (0.8-13 mg/L).

The total concentration of cocaine in blood, urine, vitreous humor, bile and stomach contents is 300.49 mg/L, 401.6 mg/L, 12.01 mg/L, 84.2 mg/L and 156.8 mg/L respectively. The relative distribution of total cocaine concentration in urine, vitreous humor and bile with respect to blood is 1.33, 0.04 and 0.28 respectively. The relative distribution of cocaine and its metabolites in various specimens (blood, urine, vitreous humor, bile and stomach contents) has demonstrated that urine and bile are good candidate for cocaine analysis. Whereas vitreous humor is owing to having less total cocaine, it is relatively less preferred specimen. Furthermore, the presence of methylecgonidine in blood indicates the victim has smoking history, as it is formed during smoking.

Conclusion: The concentrations of cocaine, BE and EME were studied in blood, urine, stomach contents, bile and vitreous humor. Altogether, the toxicological results indicate a lethal cocaine overdose in this case. This case study can be valuable piece to address the challenges associated for cocaine and its metabolite interpretation in biological specimens. In absence of anatomical or pathological conditions, the cause of death in this case can be attributed to cocaine toxicity.

The most excessive methylamphetamine antemortem blood concentration detected in a deceased newborn

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Background & Aims: Methylamphetamine detected in deceased infants has been reported in many studies. Since 2015 there have been approximately ten matters where methylamphetamine was detected in the antemortem and / or post-mortem blood samples of infants were investigated by New South Wales Police Force and / or the State Coroners. Methylamphetamine was found to have contributed to the deaths in seven cases, and methylamphetamine's toxicity was identified as the direct cause of death in only one newborn - her antemortem blood methylamphetamine concentration was 1.0 mg/L. To the best of the author's knowledge, this is the highest antemortem methylamphetamine blood concentration reported in any deceased infants. This case study aims to prevent similar deaths in the future.

Methods: A newborn female "X" was born at a gestational age of 38 weeks inside a private car that was parked at a hospital emergency bay. The umbilical cord was not immediately clamped so there was initially more blood flow between the mother and "X" than a neonate born inside hospital. Her birth weight was 3.2 kg, body length was 52 cm and head circumference was 35 cm, all of which are within the normal range. Her condition very suddenly and severely deteriorated five hours following birth. Her blood sample was collected at about one hour prior to death, the blood methylamphetamine concentration of 1.0 mg/L and amphetamine concentration of 0.07 mg/L were reported. Her central blood was collected about 48 hours after death revealing a methylamphetamine concentration of 1.1 mg/L and amphetamine concentration of 0.09 mg/L. Both samples were analyzed in the same lab by LC-MSMS technique. Years after the death of "X", her mother admitted that, about one day prior to her birth, she accidentally ingested a sip of a clear liquid that tasted bitter at a party.

Results & Discussion: The antenatal history of "X" was reported as unremarkable with a low risk on prenatal testing and a normal morphology scan about one week prior to being born. Following her birth, "X" reportedly experienced breathing difficulties and was transferred to the Special Care Nurse group where her respiratory distress had settled, and she had been actively crying. Two hours after being born the life signs of "X" were considered normal except that she had a slightly elevated heart rate and was very irritable with crying strongly which would not settle. Her breathing was regular without signs of respiratory distress.

Five hours following birth, the condition of "X" suddenly acutely deteriorated with shallow breathing, significant respiratory distress, low oxygen saturation and low heart rate. Despite active attempts at resuscitation, "X" passed away nine hours following birth. Her treating medical team were of the opinion that she was not expected to dete-

riorate so quickly and severely. At that time, it was considered the most likely cause of her acute deterioration was cardiac related pulmonary hypertension.

At the autopsy, it was noted that her heart appeared to be normal. There was nothing of significance found to assist in identifying the cause of death except for the finding of acute and chronic portal tract inflammation. This can be a possible complication of methylamphetamine use during pregnancy.

Studies revealed that the common pediatric methylamphetamine poisoning signs are tachycardia, agitation, crying, irritability and vomiting. Infants exposed to methylamphetamine are more likely to suck poorly, have a poor sleeping pattern, cry excessively, and require newborn unit admission. Reported post-mortem blood methylamphetamine concentrations detected in infants and stillborn are between 0.007 and 1.60 mg/L, with the median level less than 0.1 mg/L.

Methylamphetamine can be transferred from maternal blood to fetal blood via the placenta. The drug remains in the fetal circulatory system for much longer than in the maternal circulatory system due to the immature metabolic ability of the newborn. Maternal administration of methylamphetamine can result in an increased heart rate and an elevation in blood pressure, which can result in premature separation of the placenta from the uterine wall. This can result in spontaneous abortion or premature delivery. Acutely elevated blood pressure in the fetus can also cause fetal stroke.

Conclusion: The signs which "X" displayed are consistent with methylamphetamine's effects. The possible cause of the death of "X" as offered by her treating medical team - cardiac related pulmonary hypertension is consistent with methylamphetamine's toxicity.

The excessive methylamphetamine concentration detected in the antemortem and post-mortem blood of "X" most likely would have been due to her mother having used the drug hours prior the delivery. The methylamphetamine and amphetamine would probably have transferred via the placenta especially if the umbilical cord was not clamped immediately following delivery.

After investigation, a Coroner concluded that the direct cause of death of "X" was methylamphetamine's toxicity. Any mother of an unborn child who uses, by any method, any amount of methylamphetamine, even a small quantity, can cause lethal effects to the unborn child.

PM P-45

Sudden cardiac deaths related to QT prolonging drug use.

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Background & Aims: The heart has an electrical system of depolarization and repolarization of the ventricles that allows it to contract in a rhythm. This electrical activity is outlined on an electrocardiogram (ECG) with P waves, the QRS complex, and T waves. The QT interval clinically represents the repolarization of the ventricles. Prolonged QT is a disorder of the electrical activity, which may lead to unstable cardiac rhythm and degenerate into ventricular fibrillation causing sudden death.

In the literature there are multiple risk factors that can cause a prolongation of the QT interval and cause ventricular fibrillation, such as bradycardia, hypothyroidism/hyperthyroidism, electrolyte disorders, diabetes, congestive heart failure, renal and/or hepatic impairment, medications that prolong the QT interval, or concomitant administration of drugs that prolong the QT interval by inhibition of its metabolism.

The objective of this investigation is to study subjects who died of sudden cardiac death after the simultaneous consumption of substances that increase the QT interval.

Methods: Sudden cardiac deaths were retrieved from the database of our laboratory. We selected those in which antidepressant, antipsychotic or antiepileptic drugs were detected in the blood. Those cases that presented a concomitant consumption of drugs of abuse as well as other psychoactive substances at toxic concentrations were discarded.

Results & Discussion: 40 cases met the established criteria. 35 were males and 5 females, the age ranged from 18 to 75 years (median 49). Pregabalin (16 cases) was the most frequently implicated drug followed by quetiapine (13 cases) and methadone (12 cases). 20 QT-prolonging substances were detected in the blood. Methadone was detected in combination with sertraline, trazodone, diazepam, citalopram, quetiapine, clonazepam, mirtazapine, alprazolam, amitriptyline, tramadol, aripiprazole, bupropion, clozapine, venlafaxine. Different previous pathologies were found in the subjects like obesity, hepatitis A, hepatitis C, cardiac pathologies, hepatic and/or renal disfunction, arterial hypertension, dyslipidemia, epilepsy and toxic habits like smoking, alcoholism, or the use of drug of abuse.

Conclusion: It is important to consider the patient's clinical history before prescribing medications that may cause QT prolonging. All reported cases of sudden deaths corresponded to subjects with previous pathologies and consumption of substances that prolong QT. Another important aspect is to avoid the simultaneous prescription of substances that increase QT, as its effects could be enhanced. Methadone and citalopram are drugs that increase the QT interval and when used in combination with other drugs such as serotonin reuptake inhibitors (sertraline, trazodone, venlafaxine, tramadol) and amitriptyline, increases the risk of ventricular fibrillation and sudden death. Combining drugs use like diazepam, clonazepam, alprazolam, bupropion should also be avoided because they inhibit the metabolism of QT prolonging drugs which is a risk factor for causing ventricular fibrillation and sudden death.

Other drugs such as aripiprazole, clozapine, mirtazapine, quetiapine, and venlafaxine may cause QT prolongation but do not increase the risk of fibrillation unless used with others that will increase risk or in subjects with risk factors.

Suicidal death due to ingestion of the organophosphorus pesticide ethoprophos

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Background & Aims: Ethoprophos is an effective and widely used pesticide for controlling nemathelminths and soil insects. However, the reports on intentional, suicidal ingestion of ethoprophos (WHO hazard classification: Ia - extremely hazardous) by individuals with fatal outcomes are rare in the forensic literature.

Herein, it is reported a case of a 60-year-old man who was found dead in his vehicle by his family in a rural area of Epirus. The decedent was found lying on the front passenger's seat. The scene investigation by the police authority did not reveal any suspicious circumstances, equipment, or containers. The death was considered sudden, and the decedent was subjected to a postmortem investigation. Upon autopsy, no external injuries were observed. The decedent was wearing a T-shirt with saliva stains on the upper part. Internal examination revealed cerebral edema and pulmonary edema as the main pathological findings.

Methods: Interestingly, approximately 10 mL of mushy content in the stomach, along with multiple black granules of about 1.0 mm in diameter each, attached to the gastric mucosa, were observed. Peripheral blood, and urine specimens, as well as gastric contents, including the black granules, were collected upon autopsy for toxicological analysis.

Firstly, blood ethanol concentration was measured by an HS-GC-FID method, and screening for the presence of drugs of abuse in blood was performed by immunoassays, both with negative results. Screening for the presence of common drugs was performed by GC-MS in blood in full scan mode.

Secondly, a fraction of the black granules was isolated from the gastric content, extracted with methanol, and subjected to GC-MS screening in full scan monitoring.

Eventually, an LC-MS/MS method was developed for the quantification of the substance in blood. Experiments were carried out by a Dionex HPLC system coupled to a Q-trap 5500+, equipped with electrospray ionization (ESI) Turbo V Source, operated in multiple reaction monitoring (MRM) and in positive mode. A reversed-phase column (C18) was used, and a gradient of ACN over H2O with a total analysis time of 7.5 minutes was applied.

A quantity of 50 µL of blood, was transferred into a 2 mL Eppendorf tube, with the subsequent addition of 150 µL of acetonitrile (ACN). The sample was vigorously stirred and centrifuged (10 min, 10,000 rpm). Afterwards, 100 µL of the organic layer was taken-up and evaporated to dryness under a gentle stream of N2 (40°C). The dry residue was reconstituted with 50 µL of H2O : ACN (88:12) and injected into the LC-MS/MS system for analysis.

Results & Discussion: The organophosphate ethoprophos was identified as the main component of the granules. Ethoprophos was the only toxic substance identified in blood with GC-MS in full scan mode and confirmed by selected ion monitoring (SIM) mode. Ethanol analysis was performed in whole blood by HS-GC-FID, screening for drugs of abuse was performed by immunoassays both with negative results.

The developed UHLC-MS/MS method showed good sensitivity for ethoprophos, with limit of detection (LOD) and limit of quantification (LOQ) 1.7 and 5.6 ng/mL, respectively, and allowed the determination of ethoprophos in trace amounts both in blood and urine.

The manner and cause of death of the case was considered suicidal ingestion of the organophosphorus pesticide ethoprophos. In such cases, death occurs rapidly, as pesticides are highly toxic, even in low concentrations.

Conclusion: A simple, fast and sensitive method was developed for the determination of ethoprophos in blood using LC-MS/MS. The reported case, is a rare suicidal death due to ethoprophos ingestion.

PM P–47

The two faces of fentanyl poisoning: First cases on Spanish Mediterranean coast

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Background & Aims: Fentanyl, a potent opioid analgesic that primarily targets the μ-opioid receptor, is an important tool in the management of chronic and breakthrough pain. However, its emergence in illicit drug markets poses a significant public health threat, leading to an increase in opioid-related overdoses, including deaths.

The illicit drug landscape, particularly in North America, has seen a rapid influx of fentanyl and its analogues, exploiting existing demand for opioids such as heroin and prescription drugs. In Spain, by contrast, evidence of fentanyl-contaminated heroin and misuse of fentanyl patches for chronic pain management has been very limited to date, despite intensive surveillance.

This article presents two different cases of fentanyl poisoning in Spain to illustrate the different routes of intoxication. The first case involves a 53-year-old man found unconscious near a suspected drug outlet, reflecting the dangers of fentanyl-laced heroin in recreational drug use. In contrast, the second case involves a 31-year-old woman found unresponsive at work, highlighting the risks associated with the misuse of fentanyl patches prescribed for chronic pain

Methods: The toxicological study is based on the results of analyses of biological samples (blood and urine) by gas chromatography-single and triple quadrupole mass spectrometry and UHPLC- triple quadrupole mass spectrometry. The epidemiological study was carried out on the basis of the information provided to our laboratory by the coroner's office.

Results & Discussion: In the case of the man, several substances were found in the blood and urine (cocaine, opioids and benzodiazepines in toxic concentrations), which in combination could be lethal. In the case of the woman, a toxic concentration of fentanyl alone was found

Conclusion: Fentanyl-related deaths are so far rare in Spain. Therefore, these cases provide valuable information on the local circumstances of fentanyl poisoning, which can help to inform a comprehensive approach that includes prevention, harm reduction and treatment strategies.

PM P–48

Unsual fatal cases of carbon monoxide poisoning exposed to a leaking gas cylinder from Republic of North Macedonia in the period between 2020–2023

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Background & Aims: Carbon monoxide (CO) is an odorless, colorless, tasteless, nonirritating however highly toxic gas. CO is forming during an incomplete combustion of organic material, which include gasoline, coal, wood, propane, natural gas. Exposure to carbon monoxide was measured from blood samples expressed as a percentage of carboxyhemoglobin. Carbon monoxide has very high affinity to hemoglobin, which is about 210 times more than the oxygen. Factors that can affect the binding rate of carbon monoxide with hemoglobin in the blood are: gender, age, physical fitness, existence of acute and chronic diseases, etc.

This study describes several fatal CO poisoning cases exposed to a leaking gas cylinder from Republic of North Macedonia.

Methods: In this study were included only cases which deaths were related to CO poisoning consequently to exposition of a leaking gas cylinder in the period between 2020-2023. Toxicological findings of autopsies performed at the Institute of Forensic Medicine, Criminalistics and Medical Deontology, Medical Faculty, Ss. Cyril and Methodius University – Skopje, has been used in the study. Analysis for carboxyhemoglobin in blood were performed with Conway diffusion cells based on microdiffusion redox reaction with PdCl2. After diffusion, samples were analyzed using spectrophotometer Shimadzu UV-1800. The blood samples were protected using liquid paraffine immediately after autopsy.

Results & Discussion: Between the period of 2020-2023, there were 52 cases suspected for CO poisoning, of which 4 cases (7.7%) were a result of gas cylinder leakage. In Case No1., 70 year old man was found dead in his camper van. Inside the van there was gas cylinder tied with a stove for cooking. Case No.2 and Case No.3 were spouses in their mid-sixties, found in their bathroom. Near the bodies there was a gas cylinder. The fourth case was a 16 year old boy found in the bathroom that also had a gas cylinder inside. The screening for all cases was negative for drugs of abuse and medicines. 3 of 4 cases were negative for ethyl alcohol with exception of Case No. 1 with EtOH blood concentration of 0.33‰. The results obtained for carboxyhemoglobin are as follows: 43.84%, 43.8%, 46.96,

22.8%, which means 3 of 4 have around 40% carboxyhemoglobin. In comparison, it was a small number of cases, but it is significant because there is no installed gas supply in households in our country. CO poisoning as a result of a leaking gas cylinder has been reported in the elderly, and all cases have been detected in unventilated closed spaces.

Conclusion: People should be alarmed about the danger of using a gas cylinder in a nonventilated closed spaces. They should be aware about the danger of this silent killer. To avoid carbon monoxide poisoning when using devices connected to a gas cylinder, the concentration of carbon monoxide in the living atmosphere should be controlled by installing CO detectors.

Poster gallery – AB P-1 to P-12

10:00 – 10:30 Friday, 6th September, 2024

Ancient materia medica, modern therapeutic substances and drugs of abuse through dental calculus – ROMAN CALCULUS

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Background & Aims: Dental calculus can be defined as an oral mineralized biofilm which is sequentially generated during spontaneous calcification events. This deposit can survive through time and be detected by the most advanced technologies offering unparalleled biographical information on ancient diet, health and living condition. In the frame of a multidisciplinary project, ROMAN CALCULUS, we aim to expand the current knowledge on ancient medicine by i) analyzing Roman individuals dated from the 1st to the 6th century AD and affected by chronic oral pathological conditions requiring the use of therapeutic remedies, and ii) setting new standards and methods for identifying markers of the consumption of drugs, stimulants and/or herbal remedies, mineral and metallic substances from roman dental calculus samples. In this work, we described the study approach and methodology of this project. It was funded by PRIN PROGRAM (Research Projects of National Relevance) 2022 (CUP: B53D23002080006; funding: € 291,628.00).

Methods: Two populations will be enrolled: 1. non-consumers of any drugs of abuse and 2. consumers of drugs. The first group will provide "blank dental calculus" for methods validation; the second group will give "positive" dental calculus, hair and saliva samples. These specimens will be used for the investigation of the forensic value of tartar. The validation will be carried out following the international guidelines on forensic/toxicological analyses using gas/liquid chromatographic techniques (GC or LC) coupled to single/tandem mass spectrometry (MS or MS/MS). Besides the "classical" drugs of abuse and their main metabolites, we will expand the breadth of detectable compounds to modern medicines (i.e. benzodiazepines, antidepressants, prescription opioids) and the marker of alcohol consumption ethyl-glucuronide (EtG). For archaeological applications, substances such as Δ-9-tetrahydrocannabinol (THC) and opiates (morphine, codeine, tebaine, papaverine), will be included as present in plants used in traditional medicine and used to flavour alcoholic beverages and spirits.

Results & Discussion: In this first phase, we are developing a selective and multi-step method for quantification of opiates by LC-MS/MS. It was achieved by LLE extraction (adding dichloromethane, pH 8) of dental calculus samples ranged from 1 to 10 mg. Acquisition was performed in ESI+ and in MRM mode (2 transitions for each substance); chromatographic separation was achieved with a C18 column (2.1 ×100 mm, 1.8 µm). The method proved to be linear for calibration range of 0.005–50ng. LLOQs ranged from 0.01– 0.05 ng. Accuracy and precision were always within the acceptance criteria (%MRE≤20% and %CV ≤20%, respectively).

Conclusion: This study is the first attempt to investigate the forensic validity of dental calculus, a deposit that, until now, has been considered "waste" in forensic research. In particular, it will develop new analytical methods on contemporary dental calculus that will make this matrix a key proxy for detecting hidden markers of lifestyle, health status, diet, and living conditions. The results of the experimental protocol on modern dental calculus will be relevant for developing new methodological guidelines for forensic use of this matrix as a potential geographical and occupational marker for the secondary identification of victims. Ultimately, our application will generate new data for "reconsidering" mineralized dental plaque as a source of valid data about drug consumption habits to be compared or integrated with data obtained through standard matrixes (i.e. hair) analysis. Moreover, we expect that the results of ROMAN CALCULUS will provide a significant contribution also in clinical medicine.

AB P-02

Significance of alternative biological matrices for opioids and benzodiazepines quantification

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Background & Aims: The windows of drug detection in the keratinized matrices hair and nails is much larger compared to other biological matrices, enabling a retrospective assessment of drug consumption. Oral fluid is another non-invasive sample to assess recent drug exposure. The aim of this study was to detect and quantify benzodiazepines (BDPs), opioids (OPs) and their metabolites in samples from 27 drug offenders.

Methods: Conventional samples included blood, urine and liver, while alternative samples were hair, oral fluid and fingernails. Blood, urine and oral fluid had no pre-treatment, while liver was homogenized. Hair and fingernails were washed with dichloromethane for 5 minutes. BDPs and metabolites were alkalinized and extracted into a non-polar solvent mixture and derivatized with MTBSTFA. OPs and metabolites were extracted by solid phase extraction and derivatized with BSTFA. Analysis was performed on an Agilent GC-MS/SIM mode/DB-5 MS capillary column. Hair was treated as per Society of Hair Testing (SoHT) recommendations and the analytical method was validated using SWGTOX guidelines.

Results & Discussion: The most frequently detected drugs in conventional and alternative samples (respectively) were diazepam (n=22 vs 13), nor-diazepam (n=11 vs 8), temazepam (n=9 vs 7), oxazepam (n=11 vs 5), alprazolam (n=17 vs 5), morphine (n=22 vs 14), codeine (n=14 vs 4), nor-codeine (n=16 vs 8), and meperidine (n=8 vs 3).

Concentrations in blood, urine and liver, respectively were 5-18759 µg/L, 19-1569 µg/L and 6-339 µg/kg for diazepam; 24-61 µg/L, 31-248 µg/L and 2-65 µg/kg for nor-diazepam; 23-229 µg/L, 93-8144 µg/L and 9-33 µg/kg for oxazepam; 19-705 µg/L, 65-908 µg/L and 12-92 µg/kg for temazepam; 12-759 µg/L, 19-359 µg/L and 12-139 µg/kg for alprazolam; 84-1264 µg/L, 2153-267985 µg/L and 64-956 µg/kg for morphine; 18-159 µg/L, 3369-111849 µg/L and 6-119 µg/kg for codeine; 45-759 µg/L, 1435-162586 µg/L and 6-339 µg/kg for norcodeine; 11-64 µg/L, 43-93 µg/L and 8-23 µg/kg for meperidine for the drug offenders.

Concentrations in hair, oral fluid and fingernails, respectively were 4.2-5.2 ng/mg, 20-311 ng/mL and 0.07-0.19 ng/mg for diazepam; 0.03-1.0 ng/mg, 15-31 ng/mL and 0.04-0.84 ng/mg for nor-diazepam; 0.03 ng/mg, 0.07-2.37 ng/mL and 0.02 ng/mg for oxazepam; 0.60-1.51 ng/mg, 0.98-3.2 ng/mL and 0.17 ng/mg for temazepam; 0.04-0.09 ng/mg, 0.08-0.11 ng/mL and 0.06 ng/mg for alprazolam; 1.9-5.9 ng/mg, 367-5997 ng/mL and 4.2-15.8 ng/mg for morphine; 2.3-3.9 ng/mg, 221-498 ng/mL and 0.87 ng/mg for codeine; 1.4-2.5 ng/mg, 347-3997 ng/mL and 0.23-0.71 ng/mg for norcodeine; 0.42-0.93 ng/mg, 121-298 ng/mL and 0.07 ng/mg for meperidine for the drug offenders.

Conclusion: Unconventional matrices are of utmost significance in forensic toxicology particularly when conventional specimens are unavailable. Our findings suggest that hair and fingernails can be used as alternative specimens for chronic drug use, whereas oral fluid can be used to detect recent drug use.

AB P-03

Quantitation of an oral fluid drug panel including THC using high resolution accurate-mass (HRAM) orbitrap mass spectrometry

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Background & Aims: As labs move towards oral fluid for ease of collection and roadside testing, it is important to be able to test for a wide range of analytes and achieve required sensitivity. With the Substance Abuse and Mental Health Services Administration (SAMHSA) guidelines providing LOQ levels, the extraction protocol and instrumentation need to be sensitive enough to accomplish these cut-offs. Including tetrahydrocannabinol (THC) into the assay provides challenges in the extraction as most drugs of abuse are basic and THC is neutral. This extraction workflow, which extracts THC alongside other drugs of abuse, coupled with the Orbitrap™ mass spectrometer generates high-resolution accurate mass data that offers improved sensitivity, selectivity, and accuracy for detection and quantitation of drugs of abuse in oral fluid.

Methods: Nine calibration levels (ranging from 0.5 to 1000 ng/mL) were made by spiking stock solution of the 31 target analytes into human oral fluid. Samples were diluted with a preserving buffer and spiked with their corresponding internal standard. 500 µL of each sample were then extracted using DPX INTip™ SCX/WAX (strong cation exchange/weak anion exchange) SPE. Drug analytes were separated with on a Thermo Scientific™ Accucore™ Biphenyl column connected to a Thermo Scientific™ Vanquish™ Horizon UHPLC system using a fast 7-minute method. Data was acquired on the Thermo Scientific™ Orbitrap™ Exploris™ 120 mass spectrometer using data dependent MS2 mode (ddMS2) with an inclusion list for the 31 target drugs. Thermo Scientific™ TraceFinder™ 5.2 software was used for data acquisition and processing.

Results & Discussion: Limits of quantitation (LOQ) determined for the 31 drugs were all below the new SAMHSA guidelines cutoffs and linearity was achieved from as low as 0.5 ng/mL to a ULOL of 1,000 ng/mL. THC delta-9 achieved an LOQ of 1 ng/mL. All drugs were confirmed with mass accuracy of less than 5ppm, retention times, and library matching. This study was also able to overcome some of the issues commonly associated with THC including "stickiness" of the drug to consumables and its susceptibility to being suppressed by oral fluid collection device buffers.

Conclusion: This fast and quantitative method was developed around 31 drugs of abuse specified by SAMHSA and the National Safety Council. A complete workflow was presented that involved sample preparation using DPX INTip SPE. Linearity was achieved from LOQ's as low as 0.5 ng/mL up to 1,000 ng/mL which exemplifies the sensitivity of these instruments and extraction procedure. This fast and sensitive method for oral fluid testing sufficiently passes the SAMHSA guidelines.

Retrospective evaluation of EtG adherence analysis results in hair in the context of organ transplantation and comparison to corresponding results of urinary EtG analysis

AB P-04

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Background & Aims: 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 either performed in urine and/or hair. The aim of the presented study was the retrospective evaluation of EtG analysis in hair data obtained from January 2020 to March 2023 and to compare them to corresponding urinary ETG adherence testing results, if available.

Methods: Hair samples were analysed for EtG by external laboratories (FTC Munich or MVZ Dessau-Rosslau). EtG cut-offs were applied for classification according to the recent guidelines and recommendations of the society of hair testing, namely of 7 pg/mg or since 2022 of 5 pg/mg. EtG concentration in hair (chair) < 5 pg/mg or < 7 pg/mg were considered as negative. For chair > 5 pg/mg / 7 pg/mg but < 30 pg/mg social drinking was assumed, for chair > 30 pg/mg chronic excessive drinking (> 60 g ethanol/day) was assumed. Urine specimens were first screened for EtG using the EtG DRI kit (Thermo Fisher Scientific, Dreieich, Germany) according to manufacturer's specifications. In accordance to the guideline of the German Medical Association a cut-off of 0.5 µg/mL was applied. Positive immunoassay results (cDRI > 0.5 µg/mL) were submitted for quantitative confirmation analysis using a fully validated LC-MS/MS method (cLC-MS/MS).

Results & Discussion: EtG adherence testing was performed in n = 388 specimens. 266 (~69%) of the tested hair samples were from male patients. 233 out of 388 samples (~60%) were classified as negative. Accordingly, 155 out of 388 specimens were tested positive for EtG. For most (n = 125) of this positive group a chronic excessive ethanol intake could be assumed. The remaining 30 specimens were classified as being from social drinkers. While a similar gender ratio was found in this study in comparion to previous studies, a higher positive rate was found for EtG adherence testing in hair in comparison to EtG adherence testing in urine.

For 139 of the 388 hair samples, additional urine samples provided within 14 - 93 days prior to hair sample collection were analyzed. 96 negative hair sample results were in accordance to negative results for the corresponding urine samples. Nine samples were tested positive in both hair and urine samples. One hair sample was negative, while corresponding urine was tested positive (cLC-MS/MS = 2.5 µg/mL). Interestingly, 33 hair samples showed positive results (n= 11 social and n= 22 excessive ethanol intake), while corresponding urine samples were tested negative using the applied clincical screening cut off (cDRI > 0.5 µg/mL). Within this group, 21 times one, nine times two and three times more than tree negative urine tests were performed within a time range of 14 - 93 days. Urine was analyzed on average 47 days prior hair collection.

Conclusion: Hair analysis for EtG adherence screening in the context of transplantational medicine showed a negative rate of ~60%. Additional urine testing was performed for ~ 35% of the hair samples. Discrepancies for hair and urine results and adherence interpretation were observed in about 25% of these cases. Explanations for these findings could be that the applied cut-off value for urine samples in the clinical setting is comparatively high (5-times higher than the one applied in abstinence monitoring for regranting of driver's licences) and that the patients know they have to provide a urine sample well in advance (often weeks) and thus may adjust their drinking behaviour in the days before sampling.

Psychoactive substances analysis in wastewater and environmental risk assessment

AB P-05

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Background & Aims: Psychoactive substances have potential health and ecotoxicological risks. Water-based studies have been come into prominence for psychoactive substances monitoring besides human biological materials. Water-based studies for psychoactive substance analysis started to be made in Türkiye since 2019. Therefore, there are very limited studies on the detection of these substances in the surface waters opposed to wastewater. The aim of this study is to evaluate amphetamine (AMP), benzoylecgonine (BE), cocaine (COC), codeine (COD), 3,4-methylenedioxy-N-methylamphetamine (MDMA),morphine (MOR) and 11-nor-Δ-9-tetrahydrocannabinol-9 carboxylic acid (THC-COOH) in surface waters samples taken from Küçük Menderes (KM) River that is the most important irrigation source of the Aegean Region at four different stations in three different periods and to assess the environmental risks of psychoactive substances in the KM River.

Methods: River sampling coordinates were selected in our study based on the points monitored within the framework of the KM Watershed Protection Action Plan. The samples are coded as KM and samples stations as follows; KM-01 Beydağ, KM-02 Tire, KM-03 Selçuk and KM-04 Selçuk (4 km to the Aegion Sea). Samples were collected from these four different stations in April 2019 (1st sampling), October 2019 (2nd sampling) and April 2020 (3rd sampling). 1 L grab samples were collected in amber glass bottles from a depth of 25 cm at 4 major sampling points and transported to the Ege University,Addiction Toxicology Laboratory. Internal standards were spiked at concentration 100 ng/L each. Water samples were concentrated and filtered via a solid phase extraction (SPE) method using 3 mL disposable Oasis MCX (60 mg) cartridge. The eluents was reconstituted in 1 mL of water: methanol (75:25 v/v), and 20 µL was injected into LC–MS/MS (Castiglioni et al. 2008). The chromatographic separation of 7 substances and metabolites were successfully separated in less than 15 minutes, using Hypersil gold C18 column (13 µm; 100 cm × 2.1 mm) and gradient elution was performed with a binary mobile phase of 10 mM ammonium formate in water (solution A) and methanol (solution B). Environmental risk assessment of psychoactive substances based on calculation of Risk Quotient(RQ) for three different aquatic organisms (algae, crustaceans and fishes) according to European Medicines Agency (EMA) were evaluated.RQ is calculated as the ratio of measured environmental concentration(MEC) to predicted no-effect concentration(PNEC) [RQ=MEC/PNEC]. Environmental risks were classified by using the RQ values. Risks were classified into four classes: RQ value lower than 0.01 means no risk, RQ value between 0.01-0.1 means low risk, RQ value between 0.1-1 means medium risk, and RQ value higher than 1 means high risk (Cunha, Mendes, and Marques 2019).

Results & Discussion: Chromatographic method was validated according to Forensic Toxicology Scientific Working Group Standard Practices for Method Validation(SWGTOX). Linear calibration curves of all analytes were obtained r2 at least 0,99 and linear range was 1-1000 ng/L. High recoveries were achieved and the recovery values range between 74,2% and 95,9%. In 2019 April,AMP and BE were detected in four sampling points and COC was detected at KM-01 and KM-02 points. In 2020 April, benzoylecgonine was the only detected substances at just KM-02 and KM-03 points and other compounds were <LOD in all sampling points. A comparison between the detection rates in our study with those reported for other countries showed a significant difference in the rates estimated for COC,AMP and BE (Huerta-Fontela, Galceran, and Ventura 2007; Yao et al. 2016). Predicted no-effect concentration(PNEC) were estimated from data available in the scientific peer-reviewed literature or estimated by the ECOSAR program. Environmental risk assessment of psychoactive substances based on calculation of RQ evaluated and RQ values of AMP and BE <0.01 at all sampling points in all sampling periods, that means there were no risk for aquatic life. COC was found only first sampling period, and risk assessment for COC determined as an environmentally high risk based on its RQ value 1.22 and 1.37 at KM-01 and KM-02 sampling points. Seasonal changes and changes in drug use profiles are an important limitation for these substances at ng/L levels in surface waters

Conclusion: COC, BE and AMP that the scope of zero tolerance substance by Türkiye's laws, detected in the river waters in our study. As a new era pollutant, presence and amount of these substances in river samples is an issue that needs to be addressed. Moreover, to our knowledge, this research is the first report to investigate the presence of psychoactive substances and define the environmental risks of these substances in KM River. It is necessary to conduct new studies for more sampling times and sampling points and also to evaluate the risks that such compounds and their mixtures may pose.

AB P-06

Why has ethyl sulphate not been used as ethanol biomarker in hair analysis?

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Background & Aims: Ethyl sulphate (EtS), a direct minor ethanol metabolite, has been used for years as marker for recent alcohol consumption in urine. Being less affected by bacterial degradation due to urinary infection, EtS might even be the preferable marker compared to ethyl glucuronide (EtG). Despite that, EtS has never been used as potential marker in hair. Preliminary investigations have showed always chromatographic interferences, eventually due to cosmetic hair treatment.

Shampoos contain in general either sodium dodecyl sulphate (SDS) or sodium laureth sulphate (SLS) mixtures as washing detergents. To evaluate the possibility of hair EtS contamination by these products, several shampoos as well hair from alcohol abstinent persons and from heavy drinkers have been analysed for the presence of EtS.

Methods: A target LC-MSMS method has been developed for the detection of EtS, EtG, dodecyl sulphate, laureth sulphate as well as the C4, 6, 8, and 10 analogues of SDS. Shampoos were diluted 1:10000 with injection buffer solution. Hair was extracted after pulverisation with water using the validated EtG extraction procedure. The analytical measurement was done on a Shimadzu LC 40 equipped with a Waters HSS T3 separation column coupled to a Sciex 6500+ tandem mass spectrometer.

Results & Discussion: 40 shampoos were analysed for the presence of EtS, SDS, SLS, and its analogues. EtS could be detected in four of the tested shampoos at concentrations from 5 to 24 mg/L. EtS were also detected in all four hair samples from abstainers in concentrations from 33-80 pg/mg as well as in four of the EtG positive hair samples (from 33-130 pg/mg).

Conclusion: EtS contamination of hair by shampoos can't be avoided considering the huge variety of hair care products. These observations are analogous to fatty acid ethyl esters in hair often compromised in its use when ethanol containing hair care products have been applied.

Phosphatidylethanol as a biomarker for longitudinal follow-up of ethanol abstinence – evaluation after 4 years of implementation

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AB P-07

Background & Aims: Monitoring longitudinal ethanol abstinence is relevant in the framework of e.g. drivers' license regranting, custody and familial court, workplace testing... Phosphatidylethanol (PEth) is a valuable direct biomarker for ethanol intake that can be used for this purpose. Based on a large-scale population study, we previously developed a prediction model, that can confirm ethanol abstinence or minor ethanol intake, even when consecutive positive PEth values are available (doi:10.1111/add.15811). In addition, we also validated the use of 20 ng/mL as a suitable cut-off to conclude compatibility with ethanol abstinence or minor ethanol intake (doi:10.1093/clinchem/hvad096).

Routine assessments of PEth, primarily in the framework of drivers' license regranting, are performed in our laboratory since our large-scale population study in 2019. After 4 years of implementation, we aimed to evaluate PEth as a biomarker for longitudinal ethanol abstinence and perform an independent validation and update of the prediction model.

Methods: Data from all samples analyzed in our laboratory in the period April 2019 – February 2024 were stratified based on the following: (1) include results from subjects that are sampled under our direct supervision (group 1) and results from subject from whom the sample arrived via referral through a clinical laboratory (group 2); (2) exclude all subjects for whom less then 3 samplings were available; (3) exclude subjects for whom the time between the first and second and second and third sampling was more than 40 days. Results were evaluated for their compatibility with abstinence or minor alcohol intake according to either the application of the prediction model or the 20 ng/mL cut-off, following the hypothesis that all subjects should become abstinent from the first sampling point on (if they weren't already, based on the result from the first sampling). In addition, datasets that complied with the prediction model were used to further update the model.

Results & Discussion: Samples from 1848 unique subjects formed the basis for the data stratification (527 in group 1, 1321 in group 2). For 298 subjects more than 3 samplings were available; 163 of these fulfilled the criterium for the maximum time between samplings (40 days). Note that we recommend a maximum of 4-5 weeks between 2 samplings for longitudinal follow-up. For group 1 (n=112), 72.3% of the subjects were judged to have results compatible with abstinence, 6.3% were able to remediate by the third sampling and 21.4% did not become abstinent – of note, only 3 of these subjects retained their profile of chronic excessive alcohol use (PEth > 270 ng/mL). More than 50% of all subjects had more than 3 samplings, with ~13% (15 out of 112) eventually returning to social drinking behavior (PEth 20 – 270 ng/mL). Remarkably, for 1/3 of the subjects for whom we ran the prediction model, and for whom the result was not compatible with complete abstinence between 2 consecutive sampling points, the data did indicate compatibility with abstinence if the time between sampling and reporting back the first results was subtracted from the time period between the two samplings. The latter indicates that these subjects may have changed their drinking behavior after they received the report of their first PEth determination.

For group 2 (n=51), 51.0% of subjects were judged to have results compatible with abstinence, 13.7% were able to remediate by the third sampling and 35.3% did not become abstinent - 4 of these subjects retained their profile of chronic and excessive alcohol use. More than 50% of the subjects had more than 3 samplings with ~18% (9 out of 51) eventually returning to social drinking behavior.

For both groups of subjects, we observed that in 95% of the cases where the first two samplings were compatible with abstinence (based on the decision limit and/or following the prediction model), the third sampling was compatible with abstinence as well. If the first two samplings were not compatible with abstinence, for group 1, 100% maintained the same status, while for group 2, 31% remediated.

Results for subjects that complied with the prediction model (32 in total) were used to evaluate and update the prediction model. It was confirmed that the prediction interval did not change by adding additional data and that for none of the subjects a steeper decrease in PEth was observed compared to the original dataset. The update allows us to extend the time period in which the model is applicable from 30 to 35 days.

Conclusion: The observations made based on 4 years of experience for the use of PEth as a longitudinal biomarker for ethanol abstinence confirm its strengths. The majority of the subjects that were sampled under direct supervision of experts succeeded to be and remain abstinent, in contrast to those subjects that received their results via an electronic platform (the clinical laboratories). This highlights the added-value and/or motivation that can be found with directly supervised sampling.

AB P-08

A new LC-MS/MS method for the analysis of drugs of abuse in drug rehabilitation patients using dried blood spots: comparison with a urine immunoassay method

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Background & Aims: Dried blood spot analysis (DBS), based on capillary blood sampling by finger prick, is a rapidly emerging technique in forensic toxicology for the analysis of drugs of abuse (DoA). It offers significant advantages over standard methods based on urine sampling/immunoassay (IA). These include a less unpleasant patient/observer sampling experience, increased specificity within drug classes with no cross reactivity, and enhanced sample stability.

An LC-MS/MS (qualitative/quantitative) analytical method for the determination of 76 DoA in capillary blood samples obtained by finger prick using DBS sampling cards was developed and fully validated using ISO/IEC 17025 as a guideline. The method utilizes a fully automated DBS autosampler, linked online to the LC-MS/MS system, thus obviating the need for any sample preparation steps. In addition, the technique provides integrated, real-time, non-destructive hematocrit analysis for each DBS and real-time internal standard application.

A clinical study was undertaken to directly compare the incidence and concentrations of DoA in the blood of patients undergoing drug rehabilitation using the DBS method with those obtained using an established urine/immunoassay method.

Methods: DBS samples were obtained in a clinical setting by finger prick. Cards were allowed to dry overnight at room temperature before being delivered to the analytical laboratory. DBS cards were extracted using a fully automated DBS autosampler (CAMAG, Muttenz, CH) coupled on-line to an LC-MS/MS system (Shimadzu, Deutschland GmbH, Duisburg, DE). The DBS autosampler measured hematocrit values for each DBS non-destructively and isotopically-labelled internal standards were applied by automated sprayer utility, both in real-time prior to extracting cards.
Urine samples were analysed for DoA by immunoassay using a using a COBAS 8000 (Roche, Mannheim, DE).

Results & Discussion: From the 76 DoA in the method, 52 could be analysed quantitatively (8 inter-day precision /accuracy test). Of the 28 DoA of interest to the rehab clinic 18 could analysed quantitatively. The lower limit of detection, LOD, varied from 43ng/mL (THC) to 0.005ng/mL (Tramadol); the majority of DoA displayed a linear calibration range from 2.5 to 100 ng/mL (exception: THC 43 to 1720ng/mL). Across the entire range of analytes, the average matrix effect was ca. 70% and the average recovery was 52%. Long term storage stability at various temperatures and freeze thaw stability was analyte dependent, with citalopram displaying good stability at room temperature, whereas desalkylflurazepam was unstable even at −80°C over a period of a few weeks.

Some substances remain problematic with the DBS method: the more lipophilic benzodiazepines (e.g., Phenazepam) gave unreliable data, compared to the more polar analytes (e.g., Bromazepam). THC gave irreproducible data and had relatively high detection limits.

There was excellent agreement between the DBS and urine/IA methods for the positive (qualitative) identification DoA in patient samples. Furthermore, in many cases, where urine/IA was restricted to a substance class confirmation, DBS was able to discriminate between analytes within substance classes (for example, for the benzodiazepines, between diazepam and temazepam). For some substances (e.g., ketamine, pregabalin) not detectable using the urine/IA, DBS provided a reliable alternative. For substances analysed quantitatively, a negative trend in concentration over time could be used to confirm patient adherence to the abstinence program.

Conclusion: The DBS method described here represents a realistic alternative to traditional urine testing for the qualitative/quantitative analysis of DoA in drug rehabilitation patients. Furthermore, for a substantial majority of drugs in the method, satisfactory quantitative data could be obtained, opening the possibility of monitoring trends in drug concentrations in the blood over time. The last point may be particularly important for patients checking in to rehabilitation facilities with elevated concentration levels of DoA ín urine. DoA concentrations in DBS should provide a better indicator of abstinence during the course of therapy.

We have shown that DBS testing for DoA represents a promising alternative to the direct observation method of urine collection.

GC-NPD analysis of topiramate in capillary dried plasma

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Background & Aims: Topiramate (TPM) is a widely used anticonvulsant with applications beyond epilepsy treatment, including treatment for chemical dependency. Its impact on addiction pathways is partially understood, with hypotheses suggesting modifications to the rewarding properties and subjective perception of drugs and alcohol. Patient adherence to TPM treatment is critical for its efficacy, influenced by various factors such as patient characteristics, disease severity, therapy regimen, social support, and side effects. Monitoring response to TPM in chemical dependency treatment is challenging but essential for success. Phosphatidylethanol (PEth) quantification in blood serves as a reliable marker for monitoring alcohol abstinence.

Traditionally, TPM levels are monitored in plasma or serum samples obtained via venipuncture. However, using dried plasma spots (DPS) offers logistical advantages for therapeutic drug monitoring, overcoming issues related to hematocrit levels and blood-to-plasma partition differences. This study aimed to develop and validate a method for quantifying TPM levels in capillary DPS using gas chromatography coupled with a nitrogen and phosphorus detector (GC-NPD). The validated method was applied to monitor TPM concentrations in patients undergoing chemical dependency treatment, alongside assessing PEth levels and self-reported adherence.

Methods: Three 6 mm discs were cut from the DPS membrane and incubated with 220 µL of 0.1% bovine serum albumin at 1500 rpm for 30 minutes at 45 oC. The aqueous extract was subjected to an alkaline liquid-liquid extraction with tert-Buthyl methyl ether. The organic layer was dried in vacuum centrifugal evaporator at 45 °C. The dried extract was reconstituted with 50 µl of TMAH 0.07 Molar for flash methylation, transferred to a glass vial with insert, and 1 µL was injected into the GC-NPD system.

Chromatographic analysis was performed on a GC-NPD with a dimethylpolysiloxane NA-1 column (30 m x 0,32 mm, 1,0 µm), with oven temperature of 170 °C for 2 minutes, with a 10 °C/min ramp to 270 °C kept for 3 minutes. The method was validated based on FDA guidelines for selectivity, precision, accuracy, linearity, sensitivity, stability, and extraction yield.

The validated method was applied to monitor TPM concentrations in patients undergoing chemical dependency treatment. Venous and capillary blood samples were collected for comparison, with adherence assessed using the Brief Medication Questionnaire.

TPM quantification in venous plasma samples served as the reference for capillary DPS measurements. Chloride concentrations in DPS extracts indicated the amount of plasma sampled. PEth levels in dried blood spot (DBS) samples were quantified using ultra-high-performance liquid chromatography coupled with a triple quadrupole mass spectrometer (UHPLC-MS/MS).

Results & Discussion: The total analytical run was 14 minutes, with retention times of TPM TMAH derivative and MPPH were of, 11.2 and 13.3 minutes, respectively. Method validation demonstrated linearity in the range of 1.0 to 50 µg/ml, with a 0.99 coefficient of determination (r2) and satisfactory precision (CV% 3.62-8.29%) and accuracy (98.1%-107.7%). Although TPM recovery was incomplete (53%), sensitivity was maintained, with a lower limit of quantification (LLOQ) of 1.0 µg/mL (CV% 6.99-8.29) accuracy (105.9%). Stability testing indicated TPM stability under 4, 25, and 45 °C for up to 21 days, facilitating sample transportation without refrigeration.

The method was applied to 12 patients treated with TPM for chemical dependence. Most of the participants were male (67%), with an average age of 51 years undergoing topiramate (TPM) treatment for durations ranging from 2 to 61 months. Half of the participants were receiving TPM therapy for alcohol-related disorders, while the other half were being treated for cocaine-related disorders. Some patients had a history of cannabis and MDMA use, alongside concomitant psychiatric medications.

Analysis of chloride (Cl-) levels was crucial for correcting TPM concentrations due to the non-volumetric nature of finger prick blood collection. After correction by Cl-, TPM levels ranged from 1.7 to 13.39 µg/mL and showed a strong correlation with plasma TPM levels (rs=0.963), representing an average of 101% (ranging from 81% to 128%) of the TPM levels measured in plasma. Two patients exhibited TPM levels below the reference interval, with one patient classified as adherent to TPM treatment despite receiving a lower dose. Another patient showed potential adherence issues and later discontinued TPM therapy. One patient underwent two sample collections, showing fluctuations in adherence despite maintaining therapeutic TPM levels.

Alcohol consumption in the month preceding sampling was assessed through self-report and analysis of the PEth metabolite, indicating abstinence in most participants. However, three patients exhibited quantifiable PEth levels (45, 78.5, and 140.9 ng/ml), suggesting recent alcohol consumption despite TPM therapy.

Conclusion: The study introduces a novel technique for determining TPM concentrations in capillary DPS using a cost-effective GC-NPD system. This method offers a simpler and less invasive approach to blood collection and sensitive analysis compared to traditional methods. Notably, it is the first to compare dried capillary plasma TPM levels with venous plasma concentrations.

AB P–10

Development and validation of a capillary dried plasma spot (DPS) method for monitoring pharmacotherapy in the treatment of systemic arterial hypertension

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Background & Aims: Continuous adherence to antihypertensive medications and lifestyle changes is crucial in managing systemic arterial hypertension (SAH), yet adherence to treatment can be overlooked. Pharmacological treatment involves various drugs like ACE inhibitors, angiotensin II receptor antagonists, calcium channel blockers, beta-blockers, and diuretics, known to reduce complications and mortality among SAH patients. However, adherence to multiple medications can be challenging, leading to discontinuation. Methods to measure adherence include direct and indirect approaches, with tools like the Brief Medication Questionnaire (BMQ) and Test of Morisky-Green (TMG) being notable options despite limitations like underreporting. Direct methods like metabolite dosage and quantifying serum levels offer objective assessment. The use of dried capillary plasma spots (DPS) hasn't been explored for antihypertensive analysis, it has been used for other drugs, suggesting potential for monitoring adherence. This study aims to develop and validate a bioanalytical strategy for determining antihypertensives in DPS by UPLC-MS/MS.

Methods: DPS calibrators and quality control (QC) samples were prepared by diluting working solutions with blank plasma, and three drops were pipetted onto the HealthID® plasma separation device. Sample preparation comprises a two-step protocol. Three 6 mm discs were cut from the DPS membrane and incubated with 150 µL of 0.1% bovine serum albumin at 1500 rpm for 30 minutes at 45 °C. After homogenization, 20 µL of the extract was subtracted for chloride (Cl-) analysis and transferred 100 µL from the remaining volume to a new plastic microtube. Subsequently, the aqueous extract was subjected to protein precipitation with 20 µL of 25% trichloroacetic acid. A second extraction occurred with the addition of 500 µL of methanol and 50 µL of internal standard (atenolol-D7 and amlodipine-D4) into the microtube with the three DPS discs. The tube was incubated for 30 minutes at 1500 rpm and 45 °C, followed by 10 min ultrasound extraction. The organic layer was dried in a vacuum centrifugal evaporator at 45 °C. The dried extract was reconstituted with 100 µL of the supernatant obtained from the first step of the extraction and 100 µL of methanol. The extract was filtered, and 10 µL were injected into the UPLC-MS/MS system. The column used was the Acquity Premier BEH C18 (1.7 µm, 2.1 x 100 mm Column) at 40 °C. Elution was carried out in gradient mode with 0.1% formic acid in water and acetonitrile, from 90% A to 10% B. Ionization was conducted via electrospray source in positive mode, except for hydrochlorothiazide with polarity switching. The mass conditions for the capillary were set at 3.00 kV, with a desolvation temperature of 550 °C, gas flow rate of 850 L/Hr, and cone flow rate of 50 L/Hr. The mass-to-charge ratios and quantification transitions were as follows: Atenolol 267.2<190.0, Amlodipine 409.2<294.0, Enalaprilat 349.1<206.0, Hydrochlorothiazide (in negative mode) 296.0<296.0, and Losartan 423.2<405.2. The method was validated based on FDA guidelines for selectivity, precision, accuracy, linearity, sensitivity, stability, and extraction yield.

Results & Discussion: Total analytical run time was 10 minutes, during which no interfering peaks were observed at the same retention times in analyte-free DPS samples. The retention times were: 1.59 minutes for hydrochlorothiazide, 1.7 minutes for atenolol and atenolol-D7, 2.49 minutes for enalaprilat, 4.85 minutes for losartan, 4.46 minutes for amlodipine, and amlodipine-D4. The method demonstrated linearity within the range of 0.5 to 500 µg/mL for amlodipine and enalaprilat, and from 1.0 to 1000 µg/mL for atenolol, hydrochlorothiazide, and losartan.

The 1/x model, with an r-squared value greater than 0.99, was selected for all analytes due to its minimized sum of relative error and residues. Precision and accuracy assessments of the assay using dried capillary plasma samples yielded satisfactory results, with coefficient of variation (CV%) ranging from 4.5% to 14.6% and accuracy from 89% to 113%. The average extraction yields were 75% for amlodipine, 70% for enalaprilat, 101% for atenolol, 82% for hydrochlorothiazide, and 81% for losartan. Notably, the internal standard effectively mitigated matrix effects, with an average suppression or enhancement of ionization ranging from -14.1% to +12.9%. The analytes were stable in DPS stored at 4, 25 and 45 °C for up to 21 days (86 to 113%).

Conclusion: The study presents a novel technique for simultaneously quantifying multiple antihypertensive drugs in capillary dried plasma spots (DPS) using UPLC-MS/MS. This method provides a simpler and less invasive alternative to traditional blood collection methods, while offering sensitive analysis. Its linear range, precision, and accuracy render it suitable for pharmacokinetic studies and therapeutic drug monitoring in clinical settings. A clinical trial comparing venous plasma concentrations with DPS concentrations is currently underway

Patterns of drugs of abuse and ethyl glucuronide levels in fingernail and toenail

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AB P–11

Background & Aims: While cut-offs have been established for some compounds in hair samples when testing for drugs and alcohol markers, the interpretation of levels detected is further benefited by comparing the levels detected with those previously obtained by the testing laboratory. In the context of nail results where no cut-off levels have yet been established, the statistical compilation of previously tested samples is just as important.

This paper aims to provide information from a large representative source of results from nail samples tested for drugs and alcohol in our laboratory.

Methods: 1685 of clipped nail samples (1296 cases of fingernail and 389 cases of toenail) from medico-legal and workplace sectors were collected between 2015 and 2023 and analysed for a panel of up to 100 compounds. The analytical procedure of extraction and detection by LC-MS/MS method were validated and accredited to ISO/IEC 17025 standards. Only values exceeding the recommended cut-off for drugs and ethyl glucuronide (EtG) in hair were included in the calculations.

Results & Discussion: 46 out of 100 compounds tested were detected in at least one nail type (fingernail or toenail). Cocaine, tetrahydrocannabinol (THC) and EtG produced the highest positive rate. Examples of median levels (n) obtained in fingernails were: benzoylecgonine 0.6 ng/mg (205), EtG 33 ng/mg (193), cocaine 5.5 ng/mg (170), THC 0.82 ng/mg (156) and likewise, median levels obtained in toenails were: benzoylecgonine 0.35 ng/mg (76), THC 0.31 ng/mg (56) EtG 25.5 ng/mg (52), and cocaine 2.3 ng/mg (46).

Other drugs found included median (n) for fingernails: cannabinol 0.12 ng/mg (103), anhydroecgonine methyl ester (AEME) 1.2 ng/mg (38), amphetamine 1.1 ng/mg (33), cannabidiol 0.21 ng/mg (27), 6-acetylmorphine 4.2 ng/mg (22), morphine 1.5 ng/mg (21), codeine 0.8 ng/mg (20), heroin 2.7 ng/mg (17), ketamine 1.4 ng/mg (11) and dehydroepiandrosterone 48.5 ng/mg (6); and for toenails: cannabinol 0.10 ng/mg (36), cannabidiol 0.10 ng/mg (11), dehydroepiandrosterone 16.0 ng/mg (9), amphetamine 1.6 ng/mg (8), codeine 1.5 ng/mg (4),tramadol 12.35 (4), AEME 4.7 ng/mg (2), 6-acetylmorphine 24.35 ng/mg (2), morphine 7.8 ng/mg (2) and nordiazepam 0.06 ng/mg (2).

The results for cocaine, benzoylecgonine, AEME, amphetamine, THC and MDMA in fingernail and toenails were in the same range reported by relevant publications.

The benefit of the results of our paper is to contribute to the scientific community with the levels tested in nail of our population and add to the levels contributed by other laboratories, and perhaps enable the establishment of a more realistic cut-off of for drugs and ETG in nail samples.

Conclusion: The intralaboratory compilation of previously tested nails samples in our laboratory provides guidance levels to aid interpretation of nail samples results. In addition, the publication of similar levels by different testing laboratories, may help the establishment of relevant cut-offs for drugs and EtG in nail samples.

AB P–12

Phosphatidylethanol (PEth) detected in Dried Blood Samples (DBS) after single consumption of alcohol

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Background & Aims: Phosphatiylethanol (PEth) is a direct biomarker of ethanol used in assessment of social drinking behaviour. In recent years, studies on the determination of PEth in Dried blood spot (DBS) have increased due to the ease of sample collection, transport and storage. The aim of this study was to evaluate PEth concentrations and drinking behaviour in social drinkers by quantifying PEth 16:0/18:1 concentrations in DBS and urine samples of volunteers who consumed 0.5g/kg single controlled dose (medium dose).

Methods: A total of 5 men and 5 women aged between 22 and 44 years who declared that they were social drinkers were selected to be included in the study. These individuals were asked to abstain from alcohol for 10 days. Blood samples for complete blood count (CBC) and traditional alcohol biomarkers (ALT, AST, GGT, MCV, CDT), urine samples for analysing ethyl sulphate (EtS), and breath samples for breath alcohol level were collected from the volunteers immediately for abstinence monitoring before and after the drinking experiment. Furthermore, general health and AUDIT questionnaires were administered to these subjects. The volunteers were asked to drink wine containing 12% alcohol at an oral dose of 0.50 g/kg over a 30-min period. Subsequently, DBS, breath and urine samples were collected immediately before consumption and after consumption on 24h, 4th, 7th, 11th and 14th days. However, after the earthquakes on 6 February 2023 (Turkiye), which also affected the city we live in, the study was terminated and samples could not be collected on 11th and 14th days. One of the female participants asked to leave the study on the 2nd day of the study. PEth 16:0/18:1 in DBS samples and EtS in urine were analysed by liquid chromatography tandem mass spectrometer (LC-MS/MS).

Results & Discussion: Despite an abstinence period of at least 10 days, the mean concentration of PEth 16:0/18:1 measured in all volunteers before the controlled drinking experiment was 14.41±2.14 ng/mL. The mean concentrations of PEth 16:0/18:1 measured in DBS samples after the drinking experiment was 13.68±0.67 ng/mL. The literature suggests a cut-off value of 20 ng/mL for abstinence or low alcohol use. In the present study, there was no significant difference between PEth 16:0/18:1 concentrations on pre-drinking and post-drinking sampling days, but they were measured below 20 ng/mL. Traditional biomarker and PEth concentrations detected in volunteers participating in the study indicate that they were either low or no alcohol users. Moderate single dose alcohol intake did not change this situation.

Conclusion: The current study demonstrated that PEth 16:0/18:1 concentrations measured in DBS samples after single medium alcohol consumption do not provide information about alcohol drinking behaviour. For this reason, it is recommended that the cut off value of 20 ng/ml recommended in the literature for abstinence or low alcohol use be reduced to a lower amount. Future research with different doses of alcohol drinking is needed to determine the threshold levels of PEth as a biomarker for determining drinking habits such as abstinence, social drinking or risky alcohol consumption.

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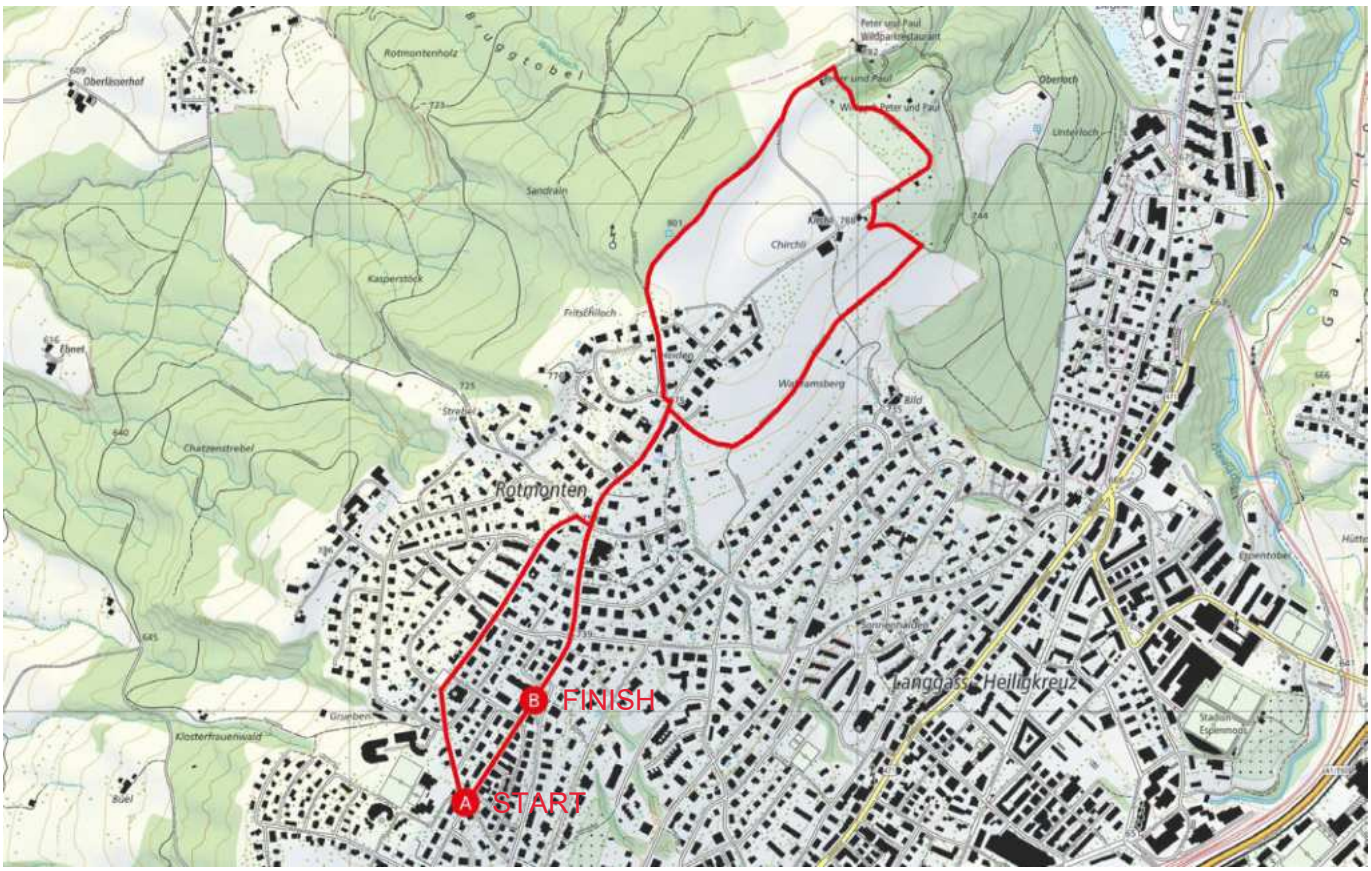
FUN RUN/WALK

Wednesday, 4th September 2024
St.Gallen

06:30 am	**A START:** bus station "Rotmonten"
07:30 – 08:00 am	**B FINISH:** coffee roastery "Beyer Beans" with coffee break until 08:00
distance:	4.3 km
altitude gain:	100 m
arrival:	bus no. 5 or 9 station "Rotmonten"
return:	bus no. 5 or 9 station "Rotmonten" or "Sonne"



GENERAL INFORMATION

Accompanying person program
Accompanying persons will have 3 day-trips included. On offer is a visit to a chocolate factory, a trip to a cheese factory and a trip up a mountain by cable car. All trips will be offered on all three scientific only days (Tuesday, Thursday and Friday). Accompanying person may select when to attend which trip at the Registration/Information Desk. However, for organizational reasons pre-booking is preferred. (Excursions for accompanying persons – The International Association of Forensic Toxicologists (tiaft2024.org)).

After conference tour offer
We offer the possibility to book a two day after conference tour "best of Switzerland" to the high mountains – check the registration page. You can always login again on the conference registration with the login and password that you created and add additional items to your booking. You can pay these separately to the primary booking.

Badge
Your personal name badge is your entrance ticket to all sessions and other activities of the TIAFT2024 Congress. Please remember to wear this badge at all times during the congress and the social activities. If you misplace you badge please consult the Registration/Information Desk for replacement.

Book Donation
Drop off your spare forensic toxicology at the Registration/Information Desk and we will pass them on to the TIAFT Book Donation Program for Developing Country Members.

Car Parking
The OLMA Messen have an underground carpark available (Sonnenstrasse 39, 9008 St.Gallen (Parkgarage Olma Messen St.Gallen)). Opening times: 24/7

Charging stations
Charging stations for mobile phones and electronic devices will be available at the conference venue.

Cloakroom
A Cloakroom will be open throughout the conference during opening hours. It will be possible to store luggage in this area.

Commercial Exhibition
A commercial exhibition will be available during the meeting. We invite and strongly encourage you to visit our exhibitors in person as well as online (Conference WebApp, link see below)

Monday, 2nd September 2024	19:00 – 21:00
Tuesday, 3rd September 2024	08:30 – 18:00
Wednesday, 4th September 2024	08:30 – 12:30
Thursday, 5th September 2024	08:30 – 18:00
Friday, 6th September 2024	08:30 – 15:00

Conference Compendium
Every delegate will receive a conference compendium upon registration containing a copy of the conference/abstract book, sponsor inserts and other items.

Conference WebApp during the event
We have a dedicated mobile friendly conference webpage. All registered participants will have received information on how to login at the beginning of August 2024. Otherwise please consult the Registration/Information Desk. The WebApp allows you to chats with fellow delegates, access the scientific program and posters if available, meet&greet the exhibitioners and check push notifications for recent updates and announcements. Don't miss it!

https://virtual.oxfordabstracts.com/#/e/tiaft2024/homepage

Currency
Swiss Francs CHF, exchange rates: 100$ = 89.6CHF, 100€ = 97.4CHF (as of 12.7.2024)

Dress code
There is no specific dress code during the conference (typically casual/smart casual). The same accounts for the gala dinner (typically business casual/cocktail), compatible with a dance party afterwards. We do have permission to party until 3 am.

Dress code Social Activity
We kindly advise those participants attending tour 2 (Lake Panorama hike on Mount Pizol) on Wednesday afternoon to wear shoes suited for light mountain hiking and to consider taking a water bottle and light rainwear (weather forecast may be found on www.meteoschweiz.admin.ch, Location: Pizol).

Duplication/Recording/Photo Policy
Unauthorized recording (audio, video, etc.) of presentations during sessions, posters, workshops, without the written consent of TIAFT and the individual authors is strictly forbidden. Attendees or exhibitors are encouraged to network end enjoy the conference experience. As such, capturing memories of casual meeting activities and networking is allowed with permission of those being prominently photographed.

Emergency Numbers
Ambulance 144, Police 117, Fire Service 118,
European Emergency Number 112, Tox-Info 145,
Air Rescue (Rega) 1414

OLMA Messen/TIAFT2024 emergency contact
+41 79 786 32 58

Excursions
On Wed Sep 4, excursions will start after a light lunch (approx. 13:00) straight from the conference venue. Delegates chose one of three different activity levels during the registration for the afternoon (Option 1: Country boattrip on Lake Constance, Option 2: Lake panorama hike on Mount Pizol, Option 3: Heidi, wine and art). We will all meet for a street food style dinner party in the country of Liechtenstein (SAL in Schaan). Busses from Liechtenstein will bring you back to St.Gallen. (Social Programm – The International Association of Forensic Toxicologists (tiaft2024.org)). Check with the Registration/Information Desk for a limited amount of spare tickets.

Flash Poster presentations
Flash poster presentations will be held in two separate locations labelled A and B in Hall 9.1 A. Check out the program.

Fun Run/Walk
On Wednesday the 4th September, we will host a fun run/walk starting at 06:30 am at the Bus stop "Rotmonten" Platform A. The run will be 4 km long with an altitude gain of 100 m. On the run you can expect spectacular views of the mountain Säntis, views down to Lake Constance, while also running through a wildlife zoo with marmots, capricorns, wild boar, lynx etc.

Food and Drinks/Catering
Water will be provided at the congress during the opening hours of the exhibition hall/congress. The congress organizer will provide catering during the coffee and lunch breaks.

House rules
The venue is a public non-smoking (including e-cigarettes) building.

Insurance and Liability
The conference Organizing Committee and Olma Messen do not accept liability for personal medical expenses/travel expenses/losses of whatever nature occurred by conference delegates.

Lost and Found
For lost and found personal items you may contact the Registration/Information Desk.

Mobile Phones/Electronic devices
As a courtesy to the speakers and fellow delegates, please switch off your mobile phones and electronic devices during sessions and presentations.

Oral presentations
Oral presentation will be held in Hall 9.1 B. Presentation time will be 9 minutes, plus 3 minutes for questions and switching between presentations. Session chairs will be instructed to be very strict regarding timing and strongly encourage questions.

Posters
All posters will be on display during the entire conference. Each poster will have a specific day/time assigned. We kindly request the author to be present and available for questions at that time. Authors will also have the possibility to upload their poster to the Conference WebApp, should they wish to share their scientific work with fellow delegates.

Pub Crawl
As we promised, we will have a TIAFT first. After the opening ceremony and the welcome reception/exhibition opening, we will hit the city of St.Gallen to showcase some of the best pubs. The local police and Dräger will support us in breath analysis, so you can scientifically test how much you had to drink. Please, refer to the flyer provided in your conference bag for details.

Questions during the Scientific Sessions
We do wish the sessions to be interactive and will foresee ways to stimulate discussion. Think pro-active about questions, don't be shy and DO ask that question!

Registration and Information Desk
At the conference venue in Hall 9.1 A. Opening times are as follows:

Monday, 2nd September 2024	08:30 – 21:00
Tuesday, 3rd September 2024	08:30 – 18:00
Wednesday, 4th September 2024	08:30 – 12:30
Thursday, 5th September 2024	08:30 – 18:00
Friday, 6th September 2024	08:30 – 15:00

Restaurants
Restaurants may be pre-booked at the Registration/ Information Desk

Scientific Program
An overview of the program can be found at (Scientific Program – The International Association of Forensic Toxicologists (tiaft2024.org)) as well as on the Conference WepApp: https://virtual.oxfordabstracts.com/#/e/tiaft2024/homepage

Speakers and Moderators
Please ensure to be available in the presentation room at least 10 minutes prior to the session. Speakers, please ensure to deliver your presentation prior to the session to the audio/ visual technicians at the AV check next to the Registration/ Information Desk in Hall 9.1 A.

Surveillance of the area
The event halls are not monitored. Please, never leave your belongings unattended.

Souvenirs
We will have a small souvenir shop available in the exhibition hall during the conference opening hours. Don't forget to take back some gifts to your home country!

Transportation/Getting Around
St.Gallen can be easily reached from Zurich airport by public transport within an hour without changing trains. You find instructions on the website (Getting there – The International Association of Forensic Toxicologists (tiaft2024.org))

Most of the locations within St.Gallen are in walking distance. However, an extensive public transport network will bring you anywhere, also until the middle of the night. If you have booked a hotel room in the city, you should get a free transportation ticket during your stay. Please, check with your hotel. Transport tickets can be purchased on all busses with bank and credits cards. We recommend buying a day pass as a return ticket has the same price as the day pass for the whole city (Zone 210, CHF 6.60)

Vendor seminars
We will have 5 vendor seminars in Hall 9.0, details maybe found on the TIAFT2024 homepage (www.tiaft2024.org) as well as the Conference WebApp.

Venue
The TIAFT2024 Conference takes place at the OLMA Messen in St.Gallen, located in the center of town. Address: Splügenstrasse 12, CH-9008 St.Gallen (https://maps.app.goo.gl/Ydg1yBRJJneWtARq5). The conference will take place in Hall 9, the Registration/Information Desk will be in Hall 9.1 A. Location/Directions to the conference venue as well as a detailed floor plan may be found on the TIAFT2024 homepage (www.tiaft2024.org).

Weather
Please check (https://www.meteoschweiz.admin.ch, Location: St.Gallen)

Wireless Internet
Wireless internet will be provided by the conference center: Network: Olma Messen Free Wifi; Detailed login instructions provided at the welcome desk

Young Scientists
The YS seminar takes place at the conference venue on Monday 2nd September, from 9-12:30 AM, followed by a lunch where Young Scientist can meet the TIAFT board (supported by TIAFT). On Tuesday September 3rd there will be a YS night out at the BrüW Pub.

Disclaimer
The organizers have made every attempt to ensure that all information in this program/Abstract book is correct. Some of the information printed has been provided by external sources. Abstracts are printed as submitted.

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