August 30th - September 4th, 2015

53rd TIAFT meeting 2015

F I R E N Z E

the city of Renaissance where Science meets Art

The International Association of Forensic Toxicologists

TIAFT

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Hee-Sun Chung

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Under the AUSPICES of

UNIVERSITÀ
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For his role in supporting this meeting – from its earliest inception to this moment – I wish to recognize FRANCESCO MARI as meeting supervisor, as my inspiration, my Maestro, my friend and, above all, my partner in life. Without him, it would not have been possible.

FRANCESCO MARI, MEETING SUPERVISOR

Research Senior Consultant, University of Florence

Promoter and founder of the Association "Group of Italian Forensic Toxicologists" in 1975.
Member also of The International Association of Forensic Toxicologists (TIAFT), the Italian Society of Legal Medicine (SIMLA), the International Academy of Legal Medicine (IALM), and the American Association of Forensic Science (AAFS).
For many years full Professor of Forensic Toxicology in the Faculty of Medicine at the University of Florence; Director of School of Specialization in Forensic Medicine; Professor of Forensic Toxicology in many degree schools, specialization schools, and masters.
He is author of over 200 scientific publications about forensic sciences and author of more than ten books on the most important topics in forensic toxicology. He is interested in the classical subjects of the forensic sciences (analytical methods, emergency toxicology, therapeutic drugs monitoring, pharmacotoxicology, pharmacodinamics of drugs of abuse, legal problems on law interpretation, behavioral toxicology, emergency toxicology) and developed a new and original research about "sewage epidemiology" in waste water plants to analyze drugs of abuse in the population of Florence, before collection in the Arno river. This method is now widely used as an epidemiological marker for drug use or abuse in populations.
He is also interested in archeo-toxicology and his research group has taken evidence of the cause of death, from arsenic poisoning, in the mortal remains of Francesco I de' Medici.
With his forensic toxicology research group at the Florence University, he took part in experimental research that led to the discovery, for the first time in humans, of the conversion of levamisole in aminorex, an important public health problem, particularly for cocaine abusers.
He continues to be the coordinator of many research projects within the Forensic Toxicology Division at the University of Florence.

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ELISABETTA BERTOL, MEETING PRESIDENT

University Professor since 1992. Full Professor of Forensic Toxicology – University of Florence from 2005.
President of Scientific Association of Italian Forensic Toxicologists (GTFI) from 2007.
Director of Forensic Toxicology Division in the Health Sciences Department from 2010, she is responsible for the Laboratory, coordinator for technical and scientific projects, and responsible for the observance of good laboratory praxis (GLP). She is highly skilled in the management of scientific instrumentation of high technology, such as gas chromatography and liquid phase chromatography, coupled with mass spectrometry and tandem mass. Since 1997 she organizes and oversees the analytical toxicological controls for the Local Commission of Driving Licenses, according to a protocol finalized for this purpose. She has managed and coordinated various research projects funded by public authorities (Regional, Ministerial, European) and private institutions (banks, foundations).
She was appointed by the Health Department of the Region of Tuscany to the Technical Working Group of the Italian Government for the preparation of procedures in workplace drug-testing; scientific collaborator with the National Early Warning System (NEWS) for drugs of abuse; and in recent years, for the identification of the NPS (New Psychoactive Substances).
According to the provisions of Italian Law on drugs, she organizes and controls the analytical procedures for the definition of administrative or criminal offences in cases of seizures of scheduled substances.
She is coordinator of the official Guidelines for the analysis of drugs of abuse in biological and non biological specimens, and is an Official Consultant of the Court of Florence, and other Courts in Italy.Scientific collaboration with :

- National Institute of Health
- Ministry of Health and Ministry of Justice
- International Institutions as EMCDDA, UNICRI , UNODC and NIDA.
- Member of National Scientific Associations: GTFI (as President)
- SIMLA, SIBIOC
- Member of International Scientific Associations : IALM, AAFS, TIAFT.

Over the years, she has participated as invited speaker and/or as chair in more than one hundred conferences, meetings and congresses in forensic toxicology, clinical toxicology, and pharmacology.
She has personally organized more than 15 Conferences and/or National Meetings of the Scientific Association of Italian Forensic Toxicologists (GTFI) at the University of Florence

Welcome Message

Dear Friends and Colleagues,

It is my great pleasure to announce that the 53rd Annual Meeting of The International Association of Forensic Toxicologists (TIAFT 2015) will be held in Firenze, Italy, August 30th – September 4th.

The chance to combine leading world experts with the unique art treasures and renaissance atmosphere of Firenze represents a valuable opportunity to promote a meeting of Art and Science and to allow people coming from all over the world to gather and exchange ideas.

TIAFT 2015 will cover all the scientific, doctrinal and technological aspects of Toxicology. We are expecting hundreds of participants from all over the world and a great contribution from exhibitors. A well calibrated program of oral and poster presentations, and dedicated workshops, will guarantee an efficient exchange of ideas and allow productive discussions.

The venue of the Meeting will be the "Firenze Fiera Congress and Exhibition Center" in the very heart of the city center and close to museums and hotels. Firenze is itself an outdoor museum, and it is easy to discover its incredible artistic patrimony just by walking through the city streets.

The social program will allow you to discover some of the most representative places of Firenze's history and environs with fruitful vineyards, modern and traditional wineries, ancient abbeys, unforgettable landscapes, and breathtaking sunsets.

I look forward to welcoming you all to Firenze!

Elisabetta Bertol
President of the Meeting

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DONATA FAVRETTO, MEETING CO-CHAIR

Donata Favretto is associate professor of Forensic Toxicology at the School of Medicine of Padova, Italy. She joined the Legal Medicine Division of the University of Padova in 2001 and she is now the Head of the Laboratory of Forensic Toxicology and Antidoping. From 1990 to 2001 she was researcher at the National Council of Researches.

She has published more than 160 papers in peer-reviewed international journals, including the first ever study of atracurium in postmortem tissues and the first ever application of MALDI-MS to hair analysis, and 6 book chapters. Her main areas of interest include drug and illicit drug intoxication, postmortem toxicology, driving under the influence, alternative specimens, ethanol markers, neonatal hair testing for documenting in-utero exposure to drugs, therapeutic drug monitoring, application of the newest technologies to forensic toxicology, implementation of the omics methodologies in forensic sciences.

She has given evidence in courts in the field of pharmacology of drugs, effects of drugs in drivers, antidoping rule violations, drugs of abuse trafficking, and has provided expert opinions in a number of cases.

She has been the scientific responsible of a unit in the European project "Integrated Project Driving under the Influence of Drugs, Alcohol and Medicines."

She has been member of TIAFT since 2001 and has actively participated to all TIAFT events since then. She was the recipient of the TIAFT award for the best published paper in 2006 in Ljubljana (Rapid Commun Mass Spectrom. 2006;20:1257-65).

She is member of the board of the Society of Hair Testing since 2012.

She is co-chair of the scientific committee of the TIAFT Meeting 2015 in Florence.

The 53rd TIAFT meeting is devoted to Irving Sunshine, one of the fathers of modern toxicology, with whom I shared science and friendship when I was a young scientist.

Irving Sunshine, PhD DABFT, DABCC (1910–2006)

"...Professional societies were served in many different ways: lectures, traveling workshops, scientific papers and journal editing, poison prevention slides and posters, committee activities and organizational chairmanships, awards from almost every toxicology organization. Most rewarding [were] the many close associations with ever so many friends. I never did a day's work in my life, it was always fun, and I am pleased to have had the privilege.—Dr. Irving Sunshine"

Dr. Sunshine's "privileged" life was shaped by his generosity in sharing his wealth of knowledge as an international teacher, scientist, mentor, scholar and orator. His lectures were always delivered with authority and a large dose of candor.

"Doc", as he was known, has been regarded as one of the founding fathers of modern-day toxicology. He was a leader in transforming toxicological analysis from early, relatively crude methods using wet chemistry to the use of sophisticated modern techniques such as spectrophotometry and gas chromatography-mass spectrometry. He mentored more than 70 postdoctoral students, trainees, and professionals from around the world. They came to learn from him for periods ranging from a few weeks to a few years. Doc would affectionately refer to these colleagues and friends as "the usual suspects", and many went on to be senior professionals in the field of toxicology. They formed a world-wide network who humorously called themselves "the Sunshine boys and girls" and who spoke affectionately and admiringly of Doc as a true friend and colleague.

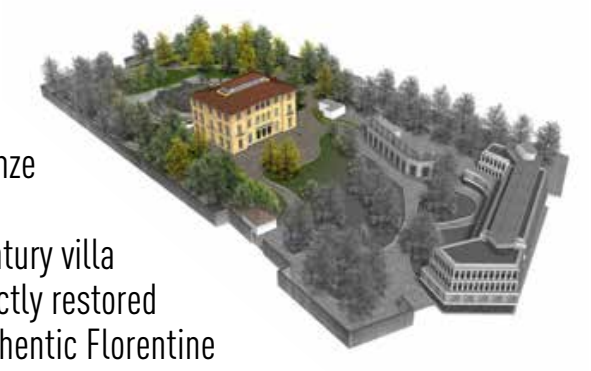
Dr. Sunshine was one of the pioneers in the Poison Control Center movement. His efforts led to the establishment of the Cleveland Poison Prevention Center, which he directed for 24 years. He was President of the American Association of Poison Control Centers from 1966 to 1968 and was Chairman of its Education Committee from 1964 to 1979.

As part of his effort in sharing with the worldwide community of toxicology, Dr. Sunshine was an inveterate international traveler. In this context, he was recognized by being made an Honorary Member of the Italian Society of Forensic Toxicologists, and he is known as the father of the Benelux Toxicology Society. In the autumn of 2005, at the age of 89, he was an invited keynote speaker at the meeting of the International Association of Forensic Toxicologists in Seoul, Korea.

In recognition of his professional distinction, devoted mentoring, and assistance to colleagues, no less than three professional societies—the International Association for Therapeutic Drug Monitoring and Clinical Toxicology, the International Association for Forensic Toxicology; and the American Academy of Forensic Sciences—established awards for distinction in their fields that are named the Irving Sunshine Award.

Cited by http://www.clinchem.org/content/53/2/373.full

Location



The TIAFT Meeting will be held at the Palazzo dei Congressi, part of the Firenze Fiera Congress and Exhibition Center.
Palazzo dei Congressi is in an 18th century villa built by the Strozzi family, today perfectly restored and furnished where possible with authentic Florentine antiques.
Palazzo dei Congressi includes several interior spaces: a prestigious auditorium which can host up to 1,000 people, an exhibition area, and other halls able to host from 50 to 200 people and located on other floors of the villa. Surrounded by a centuries-old garden, Palazzo dei Congressi and its adjacent Limonaia are easily accessible on foot from the heart of the city and the main train station.

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Organizing Committee

CHAIRS

Elisabetta Bertol
University of Firenze

Donata Favretto
University of Padova

MEMBERS

Antonina Argo (University of Palermo)
Renata Borriello (University of Napoli)
Federica Bortolotti (University of Verona)
Marina Caligara (University of Milano)
Elvira Della Casa (University of Napoli II)
Giampietro Frison (Hospital of Mestre - Venezia)
Roberto Gagliano Candela (University of Bari)
Manuela Licata (University of Modena)
Luca Morini (University of Pavia)
Cristiana Stramesi (University of Pavia)
Sabina Strano Rossi (University of Roma)
Alberto Salomone (Centro Regionale Antidoping – Orbassano - Torino)
Claudia Trignano (University of Sassari)

LOCAL MEMBERS

Laura Bigagli
Rossella Grifoni
Manuela Bomberini
Laura Magnolfi
Ester Del Bravo
Niccolò Norberti
Federica De Luca
Marco Palandri
Beatrice Defraia
Diego Palumbo
Maria Grazia Di Milia
Barbara Gualco
Alessia Fioravanti
Fabio Vaiano

Scientific Committee

CHAIRS

Donata Favretto
University of Padova

Hans Maurer
Saarland University

Franco Tagliaro
University of Verona

MEMBERS

Brice Appenzeller
Ronald Agius
Volker Auwärter
Olof Beck
Jochen Beyer
Marcus Baumgartner
Federica Bortolotti
Bertrand Brunet
Vincent Cirimele
Ana De Castro
Olaf Drummer
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Giampietro Frison
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Heike Gnann
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Marilyn Huestis
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Carmen Jurado
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Marc LeBeau
Nikolas Lemos
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Kristof Maudens
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Luca Morini
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Anna Pelander
Frank T. Peters
Sergio Pinzauti
Nele Samyn
Frank Sporkert
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Sabina Strano Rossi
Helena Teixiera
Alain Verstraete
Wolfgang Weinmann
Sarah Wille
Michel Yegles

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program

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SUNDAY, August 30th

Room 1

9.00-11.30am TIAFT Executive Board Meeting

Room ONICE

11.30-12.30pm Regional Representatives Meeting

LIMONAIA

12.30-2.00pm Lunch for TIAFT Board members and Young Scientists

Room VERDE

2.00 -4.00pm Young Scientists Meeting

Palazzo Vecchio

5.00 -7.00pm Opening Ceremony in Salone dei Cinquecento.
Welcome Message by the TIAFT President
Greetings from Local and Academic Authorities
Welcome Speech by the Meeting President
Opening Lecture by Marylin Huestis: "A conversation with Lucrezia Borgia: Saint or Sinner?"

7.00 -8.00pm Welcome Reception and Renaissance Show in Piazza della Signoria
8.00 -9.00pm Welcome Cocktail in Palazzo Borghese

MONDAY, August 31st

AUDITORIUM

8.45-9.00am **Welcome Introduction**
Donata Favretto

9.00-9.30am **First Lecture:"Addiction Neuroscience and Toxicology: a new opportunity"**
Giovanni Serpelloni

9.30-11.00am **ORAL PRESENTATION SESSION 1**
Toxicokinetics I (MOP1-7)

11.00-11.30am **Coffee Break**

11.30-1.00pm **ORAL PRESENTATION SESSION 2**
Alcohol Use Markers (MOP8-14)

1.00-2.30pm **Lunch and Poster Sessions I and II (P1-137)**

Rooms: ONICE, VERDE, 1

Company workshop

AUDITORIUM

2.30 - 4.00pm **ORAL PRESENTATION SESSION 3**
New Psychoactive Substances
(intoxication cases and identification) (MOP15-21)

4.00-4.30pm **Coffee Break**

4.30-6.00pm **ORAL PRESENTATION SESSION 4**
Toxicokinetics II (MOP22-28)

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TUESDAY, September 1st

AUDITORIUM

8.30-10.30am **ORAL PRESENTATION SESSION 1**
Driving Under the Influence I (TuOP1-8)

10.30-11.00am Coffee Break

11.00-1.00pm **ORAL PRESENTATION SESSIONS 2**
Antidoping (TuOP9-17)

1.00-2.30pm **Lunch and Poster Session I and II**

Rooms: ONICE, VERDE

Company workshop

AUDITORIUM

2.30-4.00pm **ORAL PRESENTATION SESSION 3**
Toxicokinetics III (TuOP18-24)

4.00-4.30pm Coffee Break

4.30-6.00pm **ORAL PRESENTATION SESSION 4**
Toxicokinetics IV (TuOP25-30)

Room VERDE

6.00-7.30pm Meeting + Cocktail

WEDNESDAY, September 2nd

AUDITORIUM

8.30-10.30am **ORAL PRESENTATION SESSION 1**
Driving Under the Influence II (WOP1-8)

10.30-11.00am Coffee Break

11.00-12.45am **ORAL PRESENTATION SESSION 2**
Poisoning Case Report (WOP9-16)

1.00pm Departure (with bag lunch) for Half Day Excursion

7.00pm Country Dinner in Badia a Passignano

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THURSDAY, September 3rd

AUDITORIUM

8.30-10.30am **ORAL PRESENTATION SESSION 1**
In Vivo Forensic Toxicology (ThOP1-9)

10.30-11.00am Coffee Break

11.00am-13.15pm **ORAL PRESENTATION SESSION 2**
Hair Analysis (ThOP10-18)

1.15-2.45pm **Lunch and Poster Session III and IV**

Rooms: ONICE, VERDE, 1

Company Workshop

AUDITORIUM

2.45-4.15pm **ORAL PRESENTATION SESSION 3**
New Technologies I (Th19-26)

4.15-4.30pm Coffee Break

4.30-6.00pm **ORAL PRESENTATION SESSION 4**
New Psychoactive Substances II
(Abuse Patterns and Identification) (ThOP27-32)

FRIDAY, September 4th

AUDITORIUM

8.30-10.30am	**ORAL PRESENTATION SESSION 1** **New Technologies II (FOP1-8)**
10.30-11.00am	Coffee Break
11.00-12.45am	**ORAL PRESENTATION SESSION 2** **Post Mortem Forensic Toxicology (FOP9-16)**
12.45-1.15pm	Closing Lecture by Steven B. Karch: "The Story of Hurricane Katrina"
1.15-1.30pm	Closing Ceremony
1.30-2.30pm	**Lunch and Poster Session III and IV**
2.30-4.30pm	TIAFT Business Meeting
4.30-6.30pm	GTFI Business Meeting

Palazzo Vecchio

8.30pm	Social Dinner, Awards Presentation and Entertainment in Salone dei Cinquecento

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scientific program

MONDAY, August 31st

Oral Presentation Sessions

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AUDITORIUM

8.45-9.00am **Welcome Introduction**
Donata Favretto

9.00-9.30am **FIRST LECTURE: "Addiction Neuroscience and Toxicology: a new opportunity"**
Giovanni SERPELLONI

9.30-11.00am SESSION 1 – Toxicokinetics I

Chairs: M. Meyer, N. Samyn

MOP 1 **TOXICOKINETICS OF NEW PSYCHOACTIVE SUBSTANCES: DETERMINATION OF THEIR PLASMA PROTEIN BINDING AND ITS USE IN CALCULATING METABOLIC STABILITY**
Meyer M.R.(markus.meyer@uks.eu)*, Leibnitz S., Maurer H.H.

MOP 2 **CROCODILE: METABOLISM OF DESOMORPHINE IN HUMAN LIVER MICROSOMES, HUMAN HEPATOCYTE CULTURES, AND RAT URINE AS WELL AS ITS DETECTABILITY USING STANDARD URINE SCREENING APPROACHES**
Richter L.H.J.(lilian.richter@uks.eu)*, Kaminski Y.R., Klein S., Noor F., Meyer M.R., Maurer H.H.

MOP 3 **STUDIES ON THE STABILITY OF COCAINE, MDMA, MDEA, AND THEIR MAIN HUMAN URINARY METABOLITES IN WASTEWATER BY HILIC LC-MS/MS, USING POOLED HUMAN URINE AND URINATED SOIL SAMPLES**
Mardal M.(marie.mardal@uks.eu)*, Kinyua J., Bram M., Maho W., Van Nuijs A., Covaci A., Maurer H.H., Meyer M.R.

MOP 4 **METABOLISM OF THE DESIGNER DRUG PMMA (4-METHOXYMETHAMPHETAMINE) IN HUMAN LIVER MICROSOMES**
Vevelstad M.(mevel@fhi.no)*, Øiestad E.L., Nerem E., Arnestad M., Bogen I.L.

MOP 5 **METABOLISM OF SYNTHETIC CANNABINOIDS - SIMILARITIES AND DIFFERENCES BETWEEN METABOLIC PATTERNS OF DIFFERENT STRUCTURAL SUBCLASSES**
Franz F.(florian.franz@uniklinik-freiburg.de)*, Schwörer N., Angerer V., Huppertz L.M., Moosmann B., Auwärter V.

MOP 6 **METABOLIC STUDIES OF SYNTHETIC CANNABINOID PB-22 BY CUNNINGHAMELLA ELEGANS**
Watanabe S.(Shimpei.Watanabe@uts.edu.au)*, Kuzhiumparambil U., Winiarski Z., Fu S.

MOP 7 **STUDY ON BEHAVIORAL AND PHARMACOKINETIC PROPERTIES OF 5,6-METHYLENEDIOXY-2-AMINOINDANE (MDAI) INCLUDING BIOTRANSFORMATION IN RATS AFTER A SUBCUTANEOUS BOLUS DOSE**
Zidkova M.(mzidk@lf1.cuni.cz)*, Palenicek T., Lhotkova E., Linhart I., Himl M., Balikova M.

11.30am-1.00pm SESSION 2 – Alcohol Use Markers

Chairs: A.W. Jones, L. Morini

MOP 8 **ETHYL 4-HYDROXYBUTYRATE – SEARCH FOR A LONG-TERM MARKER OF COMBINED GAMMA-BUTYROLACTONE (GBL) AND ALCOHOL UPTAKE**
Wilde M.(maurice.wilde@uniklinik-freiburg.de)*, Angerer V., Franz F., Auwärter V., Moosmann B.

MOP 9 **FORMATION AND INHIBITION OF ETHYL GLUCURONIDE AND ETHYL SULFATE**
Skopp G.(gisela.skopp@med.uni-heidelberg.de)*, Stachel N.

MOP 10 **DEVELOPMENT OF A STANDARDISED TEST FOR THE ACTIVITY OF THE ENZYME PHOSPHOLIPASE D (PLD), RESPONSIBLE FOR BIOSYNTHESIS OF THE ALCOHOL BIOMARKER PHOSPHATIDYLETHANOL (PETH)**
Schroeck A.(alexandra.schroeck@irm.unibe.ch)*, Bütikofer P., Weinmann W.

MOP 11 **OBSERVATIONS ON THE SAMPLING OF NON-VOLATILES IN EXHALED BREATH – DETECTION OF PHOSPHATIDYLETHANOL**
Beck O.(olof.beck@karolinska.se)*, Ullah S., Danielson Å., Helander A.

MOP 12 **AUTO-BREWERY SYNDROME AS A POSSIBLE EXPLANATION FOR INCREASED ALCOHOL BIOMARKERS?**
Boettcher M.(michael.boettcher@laborpraxis-dessau.de)*, Helander A., Picht F., Beck O., Boettcher--Lorenz J., Keller T.

MOP 13 **A NOVEL, SIMULTANEOUS EXTRACTION OF FAEE AND ETG FROM MECONIUM AND ANALYSIS BY LC-MS/MS**
Vaiano F.(fabio.vaiano@unifi.it)*, Favretto D., Del Bravo E., Palumbo D., Mari F., Bertol E.

MOP 14 **KINETICS OF ETG IN FINGERNAILS IN A GROUP OF CHRONIC HEAVY DRINKERS**
Morini L.(luca.morini@unipv.it)*, Hoiseth G., Giarratana N., Groppi A

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SESSION 3 – New Psychoactive Substances (intoxication cases and identification)

Chairs: M. Huestis, A. De Castro

MOP 15 **DETERMINATION OF NEWLY ENCOUNTERED DESIGNER DRUGS α-PHP AND ACETYLFENTANYL IN AN ACUTE INTOXICATION CASE BY LC/Q-TOFMS**
Hisatsune K.(hisat26@gmail.com)*, Zaitsu K., Kusano M., Yamanaka M., Nakajima J., Moriyasu T., Ishiba A., Hida M., Tsuchihashi H., Ishii A.

MOP 16 **THREE DEATHS INVOLVING SYNTHETIC CANNABINOID- PB-22 (1-PENTYL-8-QUINOLINYL ESTER-1H-INDOLE-3-CARBOXYLIC ACID)**
Pricone M.G.(mariap@vifm.org)*, Yap S., Gerostamoulos D., Drummer O.H., Woodford N.W.

MOP 17 **DEATH AFTER CONSUMPTION OF NEW SYNTHETIC CANNABINOIDS AND ALCOHOL**
Ewald A.(andreas.ewald@uks.eu)*, Schaefer N., Peters B., Schmidt P.

MOP 18 **A FATAL INTOXICATION RELATED TO MDPV AND PENTEDRONE COMBINED WITH ANTI PSYCHOTIC AND ANTIDEPRESSANT SUBSTANCES IN CYPRUS**
Liveri K.(kliveri@sgl.moh.gov.cy)*, Constantinou M., Afxentiou M., Kanari P.

MOP 19 **MEPHEDRONE IN POST-MORTEM CASES: A 5 YEAR REVIEW**
Hockenhull J.(j.hockenhull@imperial.ac.uk)*, Paterson S.

MOP 20 **DETECTION OF 31 STIMULANT, PSYCHEDELIC AND DISSOCIATIVE DESIGNER DRUGS IN REAL HAIR SAMPLES**
Salomone A., Gazzilli G., Di Corcia D., Gerace E.(enrico.gerace@antidoping.piemonte.it)*, Vincenti M.

MOP 21 **METHODS DEVELOPMENT FOR THE ASSESSMENT OF NPS USE BY ANALYSIS OF DIFFERENT BIOLOGICAL SAMPLES: HAIR, BLOOD AND URINE**
Odoardi S., Anzillotti L., Fisichella M., Strano Rossi S.(sabina.stranorossi@gmail.com)*

4.30pm-6.00pm

SESSION 4 - Toxicokinetics II

Chairs: S. Elliott, M.A. Martinez

MOP 22 **METABOLISM OF MAJOR PHYTOCANNABINOIDS BY HUMAN PLACENTA MICROSOMES AND CYP19**
Watanabe K.(k-watanabe@hokuriku-u.ac.jp)*, Inoue K., Murai M., Usami N., Yamaori S., Yamamoto I.

MOP 23 **METABOLISM OF 25I-NBOME AND 25C-NBOME, TWO SYNTHETIC HALLUCINOGENS, IN MICE AND CORRELATION WITH AUTHENTIC HUMAN URINE SAMPLES**
Wohlfarth A.(ariane.wohlfarth@gmail.com)*, Roman M., Karlsson M., Andersson M., Kugelberg F., Huestis M.A., Kronstrand R.

MOP 24 **IN VITRO METABOLISM STUDIES AND LC-MS CHARACTERIZATION OF SARM LG121071 FOR ROUTINE DOPING CONTROL**
Knoop A.(a.knoop@biochem.dshs-koeln.de)*, Krug O., Vincenti M., Schänzer W., Thevis M.

MOP 25 **INHIBITION POTENTIAL OF MDMA AND ITS RELEVANT METABOLITES ON THE IN VITRO DEAMINATION OF SEROTONIN AND DOPAMINE**
Steuer A.E., Boxler M.I.(martina.boxler@irm.uzh.ch)*, Stock L., Kraemer T.

MOP 26 **PHARMACOKINETIC MODELLING OF JWH-210, RCS-4, AND THC IN PIG SERUM AFTER INTRAVENOUS ADMINISTRATION**
Schaefer N.(nadine.schaefer@uks.eu)*, Wojtyniak J.G., Kettner M., Schlote J., Laschke M., Ewald A., Lehr T., Menger M., Maurer H., Schmidt P.

MOP 27 **THE USE OF CYTOCHROME P450 2D6 AND 2C19 GENOTYPING FOR TOXICOLOGICAL INTERPRETATION OF SPECIAL FORENSIC CASES – USEFUL OR NOT?**
Ross Jornil J.(jjor@forens.au.dk)*, Vukelic Andersen L.

MOP 28 **ENANTIOSELECTIVE PHARMACOKINETICS OF TRAMADOL AND ITS THREE MAIN METABOLITES; IMPACT OF CYP2D6 AND CYP2B6 GENOTYPE**
Haage P.(pernilla.haage@rmv.se)*, Kronstrand R., Josefsson M., Zackrisson A., Kugelberg F.C.

TUESDAY, September 1st

Oral Presentation Sessions

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AUDITORIUM

8.30-10.30am SESSION 1 – Driving Under the Influence I

Chairs: C. Stramesi, M. Baumgartner

TuOP 1 EVALUATION OF DELTA-9-TETRAHYDROCANNABINOL CONCENTRATIONS IN DUID INVESTIGATION CASES AND CONSEQUENCES OF VARIOUS THC PER SE THRESHOLDS.
Chan-Hosokawa A.(aya.hosokawa@nmslabs.com)*, Logan B.K.

TuOP 2 GHB IN SUSPECTED IMPAIRED DRIVERS IN THE NETHERLANDS AND A COMPARISON WITH POSTMORTEM CASES.
Smink B.(b.smink@nfi.minvenj.nl)*, Verschraagen M., Maes A.

TuOP 3 PREVALENCE OF NEW PSYCHOACTIVE SUBSTANCES IN DRUG DRIVERS IN SCOTLAND - A 2 YEAR REVIEW
Officer J.(jane.officer@spa.pnn.police.uk)*

TuOP 4 CRASH RISK OF MEDICINAL DRUGS
Drummer O.H. (olaf.drummer@monash.edu)*, Yap S.

TuOP 5 DRIVING UNDER THE INFLUENCE OF MARIJUANA VERSUS DRIVING AND DYING UNDER THE INFLUENCE OF MARIJUANA: A COMPARISON OF BLOOD CANNABINOID CONCENTRATIONS IN ARRESTED DRIVERS VERSUS DECEASED DRIVERS
San Nicolas A., Volk J.A., Knight J.E., Ingle E.A., Williams C.M., Lemos N.P.(nikolas.lemos@sfgov.org)*

TuOP 6 DRIVING UNDER THE INFLUENCE OF ALCOHOLAMONG CAR DRIVERS IN ISRAEL
Schallmach E.(ester.schallmach@sheba.health.gov.il)*, Gopher A., Rotenberg M.

TuOP 7 IS THC-COOH-GLUCURONIDE A USEFUL MARKER FOR TETRAHYDROCANNABINOL (THC) IN DUID CASES? RETROSPECTIVE DATA ANALYSIS ON UPLC-HR-TOFMS DATA FILES FROM 2 YEARS OF DUID CASES
Telving R.(rt@forens.au.dk)*, Hasselstrøm J.B., Andreasen M.F.

TuOP 8 DRIVING UNDER THE INFLUENCE OF ALCOHOL AND ILLICIT DRUGS. A FIVE-YEAR STUDY ON 6500 DRIVERS INVOLVED IN ROAD ACCIDENTS IN NORTH-EAST ITALY
Zancanaro F.(flavio.zancanaro@ulss12.ve.it)*, Frison G., Tedeschi G., Frasson S., Zamengo L., Zancaner S., Sciarrone R.

11.00am-1.00pm SESSION 2 - Antidoping

Chairs: P. Kintz, S. Strano Rossi

TuOP 9 LIPOSOMES AS DRUG DELIVERY SYSTEMS IN SPORT DOPING: RECENT ADVANCES IN THE STUDY OF LIPOSOME VEHICULATED HEMOGLOBINS
Colicchia S., Botre F.(francesco.botre@uniroma1.it)*, De La Torre X., Esposito S., Iannella L., Donati F.

TuOP 10 "DILUTE-AND-INJECT" MULTI-TARGET SCREENING ASSAY FOR (NOVEL) HIGHLY POLAR DOPING AGENTS USING HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY HIGH RESOLUTION/HIGH ACCURACY MASS SPECTROMETRY FOR SPORTS DRUG TESTING.
Görgens C.(c.goergens@biochem.dshs-koeln.de)*, Guddat S., Orlovius A.K., Sigmund G., Dib J., Thevis M., Schänzer W.

TuOP 11 APTAMER BASED DETECTION OF PEPTIDE AND PROTEIN DOPING THREATS IN SPORT USING GONADOTROPIN-RELEASING HORMONE AND ERYTHROPOIETIN AS MODEL COMPOUNDS
Richards S.L.(s.richards@student.unsw.edu.au)*, Cawley A.T., Raftery M.J., Cavichioli R.

TuOP 12 ISOLATION, ENRICHMENT AND DETECTION OF ERYTHROPOIETIN MIMETIC AGENTS FROM HUMAN URINE BY MEANS OF EPO-RECEPTOR-PURIFICATION AND LC-HR-MS/MS DETERMINATION
Vogel M.(m.vogel@biochem.dshs-koeln.de)*, Thomas A., Schänzer W., Thevis M.

TuOP 13 FULL-LENGTH MGF AND XENON - NEW ADDITIONS TO ANTI-DOPING TEST PROGRAMS
Thevis M.(thevis@dshs-koeln.de)*

TuOP 14 ADIPOR AGONISTS AND THEIR IMPLEMENTATION IN SPORTS DRUG TESTING
Dib J.(j.dib@biochem.dshs-koeln.de)*, Schlörer N., Schänzer W., Thevis M.

TuOP 15 MYOSTATIN INHIBITORS IN SPORTS DRUG TESTING: DETECTION OF MYOSTATIN-NEUTRALIZING ANTIBODIES IN PLASMA SAMPLES BY USING IMMUNOAFFINITY PURIFICATION AND WESTERN BLOTTING
Walpurgis K.(k.walpurgis@biochem.dshs-koeln.de)*, Thomas A., Schaenzer W., Thevis M.

TuOP 16 INNOVATIVE CHEMOMETRIC APPROACHES AS POWERFUL TOOLS IN THE ATHLETE BIOLOGICAL PASSPORT
Alladio E., Caruso R., Gerace E., Salomone A.(alberto.salomone@antidoping.piemonte.it)* Vincenti M.

TuOP 17 DRUG-DRUG INTERACTIONS AND MASKING EFFECTS IN SPORT DOPING: THE CONTRIBUTION FROM IN VITRO STUDIES COUPLED TO CHROMATOGRAPHY-MASS SPECTROMETRY
Palermo A.(amelia.palermo@uniroma1.it)*, Botre F., De La Torre X., Fiacco I., Iannone M., Mazzarino M.

53rd TIAFT meeting 2015
FIRENZE
August 30th - September 4th, 2015

2.30-4.00pm

SESSION 3 – Toxicokinetics III

Chairs: N. Lemos, F. Peters

TuOP 18 **EVALUATION OF THE BINDING AFFINITIES OF 54 NEWLY-EMERGED SYNTHETIC CANNABINOIDS AT THE CANNABINOID CB1 AND CB2 RECEPTORS**
Kikura-Hanajiri R.(kikura@nihs.go.jp)*, Uchiyama N., Hakamatsuka T.

TuOP 19 **CB1 AND CB2 RECEPTOR AFFINITIES OF SYNTHETIC CANNABINOIDS ON THE ILLICIT DRUG MARKET**
Heß C.(cohess@uni-bonn.de)*, Schoeder C., Müller C., Madea B.

TuOP 20 **CYP2D6 GENOTYPE-DEPENDING CHIRAL PHARMACOKINETICS AND PHASE I AND II METABOLISM OF MDMA AFTER CO-ADMINISTRATION OF BUPROPION IN A CONTROLLED STUDY IN HUMANS**
Steuer A.E.(andrea.steuer@irm.uzh.ch)*, Schmidhauser C., Tingelhoff E., Schmid Y., Rickli A., Liechti M.E., Kraemer T.

TuOP 21 **HEROIN KINETICS IN BLOOD AND VITREOUS HUMOR IN A LIVING PIG MODEL**
Høiseth G. (gudrun.hoiseth@fhi.no)*, Gottås A., Arnestad M., Halvorsen S., Bachs L.

TuOP 22 **SEROTONIN TOXICITY AND CYTOCHROME P450 GENETIC POLYMORPHISMS**
Gunja N.(naren.gunja@sydney.edu.au)*, Mann G., Piatkov I.

TuOP 23 **FIRST METABOLIC PROFILE OF PV8, A NOVEL SYNTHETIC CATHINONE, BY HIGH-RESOLUTION MASS SPECTROMETRY (HRMS)**
Swortwood M.(madeleine.swortwood@nih.gov)*, Ellefsen K., Wohlfarth A., Concheiro M., Huestis M.

TuOP 24 **FLUBROMAZOLAM - CHARACTERIZATION AND BASIC PHARMACOKINETIC EVALUATION OF A HIGHLY POTENT DESIGNER BENZODIAZEPINE**
Huppertz L.(laura.huppertz@uniklinik-freiburg.de)*, Franz F., Bisel P., Moosmann B., Auwärter V.

4.30-6.00pm

SESSION 4 – Toxicokinetics IV

Chairs: A. Verstraete, T. Gunnar

TuOP 25 **SEQUENCING CYP2D6 IN POST-MORTEM BLOOD SAMPLES WITH HIGH CONCENTRATION OF TRAMADOL FOR THE DETECTION OF *3, *4, *6, *8, *10 AND *12 ALLELES**
Fonseca S.(sfonseca@dlinml.mj.pt)*, Amorim A., Afonso Costa H., Porto M.J., Franco J., Costa Santos J., Dias M.

TuOP 26 **POSTMORTEM IDENTIFICATION OF METABOLIZER TYPE: A PROTEOMICS APPROACH**
Desharnais B.(brigitte.desharnais@msp.gouv.qc.ca)*, Mireault P., Skinner C.D.

TuOP 27 **CANNABINOID PHARMACOKINETIC AND BEHAVIOURAL PROFILES AFTER DIFFERENT ROUTES OF ADMINISTRATION IN RATS**
Hložek T.(tomas.hlozek@vfn.cz)*, Balíková M., Pálenícek T., Lhotková E., Kaderábek L., Tylš F., Šíchová K., Štefková K., Uttl L.

TuOP 28 **CONTROLLED VAPORIZED CANNABIS, WITH AND WITHOUT ALCOHOL: SUBJECTIVE EFFECTS AND BLOOD CANNABINOID DISPOSITION**
Hartman R.L.(rebecca.hartman@nih.gov)*, Brown T.L., Milavetz G., Spurgin A., Gorelick D.A., Gaffney G., Huestis M.A.

TuOP 29 **GHB AND ITS NEWLY DESCRIBED METABOLITE GHB-β-O-D-GLUCURONIDE – DISCRIMINATION EXOGENOUS/ENDOGENOUS VIA LC/MS/MS AND GC/C/IRMS**
Mehling L.M.(lena-maria.mehling@ukb.uni-bonn.de)*, Lott S., Maas A., Madea B., Mußhoff F., Piper T., Thevis M., Spottke A., Heß C.

TuOP 30 **BEHAVIOURAL AND PHARMACOLOGICAL CHARACTERIZATION OF A NOVEL CANNABIMIMETIC ADAMANTANE-DERIVED INDOLE, APICA, AND CONSIDERATIONS ON THE POSSIBLE MISUSE AS A PSYCHOTROPIC SPICE ABUSE, IN C57BL/6J MICE**
Malta G.(ginevramalta@gmail.com)*, Argo A., Brancato A., Roda G., Casagni E., Gambaro V., Procaccianti P., Cannizzaro C.

AUDITORIUM

8.30-10.30am SESSION 1 – Driving Under the Influence II

Chairs: C. Moore, W. Weinmann

WOP 1 **COMPARISON OF ILLICIT DRUG CONCENTRATIONS IN PLASMA AFTER ROADSIDE TESTING IN URINE OR ORAL FLUID IN THE BELGIAN DRIVING POPULATION**
Van Der Linden T., Wille S.M.(Sarah.Wille@just.fgov.be)*, Ramirez Fernandez M.D.M., Verstraete A.G., Samyn N.

WOP 2 **APPLICATION OF A LC-MS/MS METHOD FOR SELECTED SYNTHETIC CATHINONES AND PIPERAZINES TO DRIVERS' ORAL FLUID SPECIMENS**
De Castro A.(ana.decastro@usc.es)*, Lendoiro E., Cruz A., López--Rivadulla M.

WOP 3 **ULTRA-RAPID TARGETED ANALYSIS OF 40 DRUGS OF ABUSE IN ORAL FLUID BY LC-MS/MS**
Di Rago M.(matthew.dirago@vifm.org)*, Chu M., Gerostamoulos D., Drummer O.H.

WOP 4 **TIME LAPSE PERIOD OF POSITIVITY OF ORAL-FLUID TEST IN CHRONIC AND OCCASIONAL CONSUMERS AFTER ADMINISTRATION OF 10 OR 30 MG OF SMOKED THC VERSUS PLACEBO; CORRELATION WITH BLOOD CONCENTRATIONS**
Alvarez J.C.(jean-claude.alvarez@rpc.aphp.fr)*, Larabi I.A., Ribot M., Mayer C., Knapp A., Quera Salva M.A., Hartley S.

WOP 5 **OPTIMIZATION OF AN AUTOMATED SOLID PHASE EXTRACTION TO DETERMINE DRUGS IN PRESERVED ORAL FLUID USING ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY**
Ingels A.S.(ann-sofie.ingels@just.fgov.be)*, Ramirez Fernandez M.D.M., Di Fazio V., Wille S.M., Samyn N.

WOP 6 **USE OF PSYCHOACTIVE SUBSTANCES AMONG TRUCK DRIVERS IN THE HIGHWAYS OF THE STATE OF SAO PAULO, BRAZIL**
Sinagawa D.M.(daniele.mayumi.sinagawa@gmail.com)*, Takitane J., Bombana H.S., Carvalho H.B., Yonamine M., Rohlfs W.J.C., Prado N.V., Oliveira K.B.G., Muñoz D.R., Leyton V.

WOP 7 **CONFIRMATORY AND COMPARATIVE DRUG ANALYSIS OF ORAL FLUID / BLOOD SPECIMENS FROM DRIVERS**
Stoykova S.(sstoykova@chem.uni-sofia.bg)*, Atanasov V.

WOP 8 **ASSOCIATION BETWEEN ELEVATED HAIR ETG AND ALCOHOL RELATED TRAFFIC DEATHS**
Gottardo R.(rossella.gottardo@univr.it)*, Sorio D., Bertaso A., Tagliaro F.

11.00-12.45pm SESSION 2 – Poisoning Case Reports

Chairs: K. Maudens, G. Frison

WOP 9 **DATURA AND HALLUCINATIONS: THE ATROPINE RISK**
Lemaire Hurtel A.S.(hurtel.anne-sophie@chu-amiens.fr)*, Bodeau S., Bennis Y., Knapp A., Mayer C., Alvarez J.C.

WOP 10 **SUICIDE OF A NURSE IN A HOSPITAL ENVIRONMENT INVOLVING ANESTHETIC DRUGS**
Aknouche F.(aknouche.frederic@wanadoo.fr)*

WOP 11 **POISONOUS PLANTS AND ANCIENT HUNTERS: AN ANALYTICAL INVESTIGATION INTO THE PRESENCE OF PLANT ALKALOIDS ON HUNTING TOOLS FROM INTERNATIONAL MUSEUM COLLECTIONS**
Carlin M.G.(m.carlin@northumbria.ac.uk)*, Borgia V., Bowerbank S.

WOP 12 **A HIDDEN KILLER?: PREGABALIN IN FATALITIES**
Elliott S.(simon.elliott@roarforensics.com)*

WOP 13 **ETHYLPHENIDATE IN POST MORTEM CASES**
Morley S.(steve.r.morley@uhl-tr.nhs.uk)*, Smith P., Cole R., Hikin L.

WOP 14 **DEATHS INVOLVING 'DOCTOR SHOPPING' AND THE MISUSE OF PRESCRIPTION DRUGS IN AUSTRALIA**
Pilgrim J.(jennifer.pilgrim@monash.edu)*, Dorward R., Dobbin M., Drummer O.H.

WOP 15 **PAEDIATRIC DEATHS IN AUSTRALIA INVOLVING DIRECT DRUG TOXICITY 2003 - 2013**
Jenkins E.(elizabeth.jenkins@vifm.org)*, Gerostamoulos D., Caldicott D., Drummer O.H., Pilgrim J.

WOP 16 **REPORTING A DEATH INVOLVING OPIUM CONSUMPTION IN A LEGAL POPPY FIELD IN SPAIN**
MartÍnez M.A.(mariaantonia.martinez@justicia.es)*, Ballesteros S., Almarza E., Garijo J.

53rd TIAFT meeting 2015
FIRENZE
August 30th - September 4th, 2015

THURSDAY, September 3rd

Oral Presentation Sessions

AUDITORIUM

8.30-10.30am SESSION 1 – In vivo Forensic Toxicology

Chairs: E. Cone, B. Brunet

ThOP 1 ACUTE INTOXICATION WITH CANNABIS IN A 12-MONTH-OLD GIRL: UNEXPECTED HIGH CONCENTRATIONS OF THC AND THC-COOH
Jurado C.(carmen.jurado@justicia.es)*, Huertas T., Garcia S., Lopez--Oriol L., Moreno E.

ThOP 2 PASSIVE INHALATION OF CANNABIS SMOKE IS DRUG ADMINISTRATION
Cone E.(edwardjcone@gmail.com)*, Bigelow G., Herrmann E., Mitchell J., Lodico C., Flegel R., Vandrey R.

ThOP 3 LIFE-THREATENING AMLODIPINE POISONING: THE RESPECTIVE ROLE OF VENO-ARTERIAL ECMO, LIPID RESCUE AND MARS© BASED ON THE ANALYSIS OF PLASMA AMLODIPINE PHARMACOKINETICS
Soichot M.(marion.soichot@aphp.fr)*, Voicu S., Vodovar D., Deye N., Mégarbane B., Bourgogne E.

ThOP 4 USE OF BOTH THC-COOH FREE AND GLUCURONIDE TO ASSESS FREQUENCY OF CANNABIS CONSUMPTION
Hädener M.(marianne.haedener@irm.unibe.ch)*, König S., Weinmann W., Giroud C., Martin Fabritius M.

ThOP 5 WHEN ECSTASY TURNS OUT TO BE CLOZAPINE AT A DANCE EVENT...
Patteet L.(lisbeth.patteet@uantwerp.be)*, Maudens K., Wille S., Blanckaert P., Hugo N., Calle P.

ThOP 6 DETECTION OF 3-METHYLMETHCATHINONE, PAROXETINE, AND CLOTIAZEPAM IN HEAD HAIR AND/OR PUBIC HAIR SAMPLES BY LC/HR-MS ANALYSIS IN THREE DISTINCT FORENSIC TOXICOLOGY CASES
Frison G.(giampietro.frison@ulss12.ve.it)*, Frasson S., Zancanaro F., Tedeschi G., Sciarrone R.

ThOP 7 A PROSPECTIVE PILOT STUDY OF MEASUREMENT OF BLOOD CONCENTRATIONS OF ROUTINE MEDICATIONS IN PATIENTS TREATED IN THE INTENSIVE CARE UNIT
Kugelberg F.C.(fredrik.kugelberg@liu.se)*, Johansson A., Roman M., Kronstrand R., Lennborn U., Sandler H., Ahlner J., Rubertsson S.

ThOP 8 EFFECTS OF RECENTLY-EMERGED SYNTHETIC CANNABINOIDS ON LOCOMOTOR ACTIVITY IN MICE
Uchiyama N.(nuchiyama@nihs.go.jp)*, Aritake K., Kikura--Hanajiri R., Hakamatsuka T., Urade Y.

ThOP 9 DRUG SCREENING AND ILLICIT DRUG USE IN A TWO-SITE SOLID ORGAN TRANSPLANT PRACTICE
Snozek C.(snozek.christine@mayo.edu)*, Langman L., Jannetto P.

11.00am-1.15pm SESSION 2 – Hair Analysis

Chairs: C. Jurado, L. Labat

ThOP 10 HYDROMORPHONE IN HAIR: AN INDICATOR OF HEROIN INTAKE VS CONTAMINATION?
Baumgartner M.R.(markus.baumgartner@irm.uzh.ch)*, Madry M.M., Bosshard M.M., Kraemer T.

ThOP 11 UTILITY AND ALTERNATIVES OF COSMETICALLY TREATED HAIR FOR THE SENSITIVE DETECTION OF DRUGS AND ALCOHOL
Agius R.(ragius@laborkrone.de)*, Dufaux B., Nadulski T., Kahl H.G.

ThOP 12 ESTABLISHMENT OF A DIAGNOSTIC PROBE FOR THE DISCRIMINATION OF EXOGENOUS GHB IN HUMAN HAIR
Paradis--Tanguay L., Gilbert N., Vo Duy S., Sauvé S., Lajeunesse A.(Andre.Lajeunesse@uqtr.ca)*

ThOP 13 MASS SPECTROMETRY IMAGING FOR DEPICTING DRUG INCORPORATION INTO HAIR
Miki A.(Mikimickey@nifty.ne.jp)*, Kamata T., Shima N., Sasaki K., Katagi M., Sato T., Tsuchihashi H.

ThOP 14 LONGITUDINAL SCANNING OF DRUGS OF ABUSE IN HAIR USING DIRECT ANALYSIS IN REAL TIME (DART) HIGH RESOLUTION MASS SPECTROMETRY
Nielen M.(michel.nielen@wur.nl)*, Duvivier W., Van Beek T., Sterk S.

ThOP 15 OCCUPATIONAL EXPOSURE TO KETAMINE DETECTED BY HAIR ANALYSIS: A RETROSPECTIVE AND PROSPECTIVE STUDY
Favretto D.(donata.favretto@unipd.it)*, Snenghi R., Vogliardi S., Donà A., El Mazloum R., Simoncello I., Ferrara S.D.

ThOP 16 WHEN KISSING CAN RESULT IN AN ADVERSE ANALYTICAL FINDING DURING DOPING CONTROL: ABOUT 2 CASES WHERE HAIR TESTING WAS DETERMINANT FOR THE ATHLETE
Kintz P.(pascal.kintz@wanadoo.fr)*

ThOP 17 DETERMINATION OF HYDROXY METABOLITES OF COCAINE IN HAIR SAMPLES FOR PROOF OF DEFINITE CONSUMPTION
Franz T.(t.franz@ftc-munich.de)*, Steinmetz S., Dame T., Schwarz G., Musshof F.

ThOP 18 INFLUENCE OF THERMAL HAIR STRAIGHTENING ON COCAINE IN HAIR
Ettlinger J., Yegles M.(michel.yegles@lns.etat.lu)*

2.45-4.15pm

SESSION 3 – New Technologies I

Chairs: S. Pinzauti, T. Kraemer

ThOP 19 **IDENTIFICATION OF NEW PSYCHOACTIVE SUBSTANCES AND THEIR METABOLITES USING HIGH RESOLUTION MASS SPECTROMETRY FOLLOWING A NOVEL STRUCTURED WORKFLOW**
Kinyua J.(juliet.kinyua@uantwerpen.be)*, Maudens K., Negreira N., Brabanter N.D., Lanckmans K., Covaci A., Van Nuijs A.

ThOP 20 **RAPID DETERMINATION OF NEW PSYCHOACTIVE SUBSTANCES IN BIOLOGICAL MATRICES USING AN AUTOMATED IN-LINE ITSP(TM) SPE-LC-MS/MS-SYSTEM**
Lehmann S.(sabrina.lehmann@uk-koeln.de)*, Kieliba T., Beike J., Rothschild M.A., Mercer--Chalmers--Bender K.

ThOP 21 **QUANTIFICATION OF DESMETHYL METABOLITES BY UPLC WITH CORONA CHARGED AEROSOL DETECTION USING THE PARENT DRUG FOR CALIBRATION**
Viinamäki J.(jenni.viinamaki@helsinki.fi)*, Ojanperä I.

ThOP 22 **COCAINE AND METABOLITES CONCENTRATIONS IN DRIED BLOOD SPOTS AND VENOUS BLOOD AFTER CONTROLLED INTRAVENOUS COCAINE ADMINISTRATION**
Ellefsen K.(kayla.ellefsen@nih.gov)*, Luiz Da Costa J., Concheiro M., Anizan S., Barnes A., Pirard S., Gorelick D., Huestis M.

ThOP 23 **SUPPORTED LIQUID EXTRACTION (SLE) FOR THE ANALYSIS OF METHYLAMPHETAMINE, METHYLENEDIOXYMETHYLAMPHETAMINE AND DELTA-9-TETRAHYDROCANNABINOL IN ORAL FLUID AND BLOOD**
Rositano J., Harpas P., Kostakis C., Scott T.(tim.scott@sa.gov.au)*

ThOP 24 **SIMULTANEOUS DETERMINATION OF 40 NOVEL PSYCHOACTIVE STIMULANTS IN URINE BY LIQUID CHROMATOGRAPHY HIGH-RESOLUTION MASS SPECTROMETRY AND LIBRARY MATCHING**
Concheiro M.(mconcheiro-guisan@jjay.cuny.edu)*, Castaneto M., Kronstrand R., Huestis M.A.

ThOP 25 **DEFINITIVE MULTI-ANALYTE DRUG TESTING IN URINE BY UPLC-MS/MS: AN ALTERNATIVE TO PRESUMPTIVE SCREENING BY IMMUNOASSAY**
Rosano T.(rosanot@mail.amc.edu)*, Ohouo P., Leque J., Freeto S., Wood M.

ThOP 26 **DETERMINATION OF MEDICAL AND ILLICIT DRUGS IN POST MORTEM DENTAL HARD TISSUES AND COMPARISON WITH ANALYTICAL RESULTS FOR BODY FLUIDS AND HAIR SAMPLES**
Klima M.(miriam.klima@uniklinik-freiburg.de)*, Altenburger M.J., Kempf J., Auwärter V., Neukamm M.A.

4.30-6.00pm

SESSION 4 – New Psychoactive Substances II (abuse patterns and identification)

Chairs: F. Bortolotti, J. Beyer

ThOP 27 **DESIGNER STIMULANTS - EVOLVING ABUSE PATTERNS, 2011-2015**
Uralets V.(vuralets@redwoodtoxicology.com)*, Rana S., Ross W.

ThOP 28 **TRENDS AND CHANGES IN THE SYNTHETIC CANNABINOID DRUGS MARKET IN ISRAEL BETWEEN 2010-2014**
Tepperberg M., Schallmach E.(Ester.scallmach@sheba.health.gov.il)*, Rotenberg M.

ThOP 29 **NEW PSYCHOACTIVE SUBSTANCES: THE PERKS AND PITFALLS OF ON-LINE PURCHASING**
Button J.(jenny.button@chiron.no)*, Johansen J.E., Eriksson S., Liu H.

ThOP 30 **FAST IDENTIFICATION OF NEW PSYCHOACTIVE SUBSTANCES IN COLLABORATION WITH EUROPEAN CUSTOMS**
Lobo Vicente J.(joana.lobo@ec.europa.eu)*, Chassaigne H., Reniero F., Holland V., Kolar K., Guillou C.

ThOP 31 **IN SILICO PREDICTION OF AND IN VITRO METABOLISM STUDIES OF NEW DESIGNER DRUGS 25I-NBOME AND 25I-NBOH**
Nielsen L.M.(line.marie.nielsen@sund.ku.dk)*, Holm N.B., Leth-Petersen S., Kristensen J.L., Gabel--Jensen C., Olsen L., Linnet K.

ThOP 32 **25I-NBOME - CASE REPORT AND METABOLISM**
Grumann C.(christina.grumann@uniklinik-freiburg.de)*, Franz F., Hermanns--Clausen M., Kithinji J., Auwärter V.

53rd TIAFT meeting 2015
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FRIDAY, September 4th

Oral Presentation Sessions

AUDITORIUM

8.30-10.30am SESSION 1 - New Technologies II

Chairs: I. Ojampera, R. Kronstrand

FOP 1 **BRAIN DISTRIBUTION STUDIES OF ANTIPSYCHOTIC DRUG IN MOUSE WITH MALDI-TOF MS IMAGING: HALOPERIDOL**
Igarashi K.(kigarashi1868@hotmail.com)*, Yoshida M., Nakanishi T.

FOP 2 **A MULTICOMPONENT UPLC-Q-TOF METHOD FOR SCREENING OF PHARMACEUTICAL COMPOUNDS IN POST MORTEM BLOOD- VALIDATION, DATA MINING STRATEGIES AND RESULTS FROM ROUTINE ANALYSIS.**
Liane V.H.(veronicah.liane@fhi.no)*, Svendsen K.O., Kristoffersen L.

FOP 3 **HIGH SENSITIVITY/SPECIFICITY DETERMINATION OF CARBOHYDRATE-DEFICIENT TRANSFERRIN (CDT) BY A NEW METHOD BASED ON HPLC SEPARATION WITH FLUORESCENCE DETECTION**
Sorio D., De Palo E.F., Bortolotti F., Tagliaro F.(franco.tagliaro@univr.it)*

FOP 4 **ORBITRAP-BASED LC-HR-MS/MS STANDARD URINE SCREENING APPROACH: TRANSFER TO BLOOD PLASMA EXEMPLIFIED FOR CARDIOVASCULAR DRUGS**
Helfer A.G.(andreas.helfer@uks.eu)*, Michely J.A., Weber A.A., Meyer M.R., Maurer H.H.

FOP 5 **UPLC-MS/MS METHOD FOR THE SIMULTANEOUS DETERMINATION OF 35 SUBSTANCES INCLUDING MEDICINES AND DRUGS OF ABUSE ON DRIED BLOOD SPOTS APPLIED IN FORENSIC TOXICOLOGY**
Simões S.(ssimoes@dlinml.mj.pt)*, Castañera A., Franco J.M., Dias M.J.

FOP 6 **MICROWAVE-ASSISTED ON-SPOT DERIVATIZATION FOR THE GC-MS BASED DETERMINATION OF POLAR LOW MOLECULAR WEIGHT MOLECULES -AMONGST WHICH GHB AND BHB- IN DRIED BLOOD SPOTS**
Sadones N.(Nele.Sadones@UGent.be)*, Ghevel S., Lambert W.E., Stove C.P.

FOP 7 **DETERMINATION OF SYNACTHEN® IN DRIED BLOOD SPOTS FOR DOPING CONTROL ANALYSIS USING LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY**
Tretzel L.(l.tretzel@biochem.dshs-koeln.de)*, Thomas A., Geyer H., Delahaut P., Schaenzer W., Thevis M.

FOP 8 **DEVELOPMENT OF A WORKFLOW FOR NON-TARGETED HIGH RESOLUTION ACCURATE MASS SPECTROMETRY ANALYSIS IN FORENSIC TOXICOLOGY: A CASE STUDY IN NBOME DETECTION**
Cawley A.(acawley@racingnsw.com.au)*, Ganbat N., Ennis L., Smart C., Greer C., Keledjian J., Pasin D., Fu S., Chen A.

11.00am-12.45pm SESSION 2 – Post Mortem Forensic Toxicology

Chairs: A. Argo, H. Teixeira

FOP 9 **A CLUSTER OF PARA-METHOXY-METHAMPHETAMINE (PMMA) RELATED FATALATIES**
Kronstrand R.(robert.kronstrand@rmv.se)*, Lindstedt D., Roman M., Thelander G.

FOP 10 **THE GAS PROJECT : POSTMORTEM IMAGING AND ANALYSIS OF CARDIAC GASES HELP TO DIAGNOSE THE CAUSE OF DEATH**
Varlet V.(vincent.varlet@chuv.ch)*, Smith F., Giuliani N., Egger C., Dominguez A., Chevallier C., Augsburger M., Grabherr S.

FOP 11 **DETECTION OF PSYCHOACTIVE SUBSTANCES ON HAIR AND MUSCLE SAMPLES AFTER 30 YEARS BY LC-MS/MS AND MALDI-LTQ ORBITRAP TECHNOLOGY WITH DRIED MATRIX SPOT SAMPLING**
Grata E., Sporkert F., Thomas A., Déglon J., Mottini N., Augsburger M., Pinorini M.T.(maria.teresa.pinorini@fasv.ch)*

FOP 12 **THE SEARCH FOR A VOLATILE HUMAN SPECIFIC MARKER IN THE POST MORTEM DECOMPOSITION PROCESS**
Rosier E.(elien.rosier@pharm.kuleuven.be)*, Loix S., Develter W., Van de Voorde W., Tytgat J., Cuypers E.

FOP 13 **QUETIAPINE IN BRAIN TISSUE**
Skov L.(louise.skov@sund.ku.dk)*, Stybe Johansen S., Linnet K.

FOP 14 **AUTOPSY AND TOXICOLOGY FINDINGS IN THREE CASES OF INTRAVENOUS INJECTION OF ORAL TABLETS**
Ritchey D.(don.ritchey@dhhs.tas.gov.au)*

FOP 15 **FATAL COMBINATION WITH 3-METHYLMETHCATHINONE (3-MMC) AND GAMMA-HYDROXYBUTYRIC ACID (GHB)**
Jamey C.(c.jam@laposte.net)*, Kintz P., Martrille L., Humbert L., Raul J.S.

FOP 16 **TIME-DEPENDENT POSTMORTEM REDISTRIBUTION OF CENTRALLY ACTING SUBSTANCES**
Staeheli S.N.(sandra.staeheli@irm.uzh.ch)*, Gascho D., Fornaro J., Laberke P., Steuer A.E., Kraemer T.

CLOSING LECTURE: "The Story of Hurricane Katrina"
Steven B. Karch

Poster Presentation Sessions

First Group (Aug 31st – Sept 1st)

P1. HIGH SENSITIVITY ANALYSIS OF OPIOIDS IN ORAL FLUID USING IONKEY/MS
Roman G.(gregory_roman@waters.com)*, Lee R., Murphy J., Michelle W.

P2 ANALYTICAL STRATEGY FOR THE DETECTION OF SUBITRAMINE IN DIETARY SUPPLEMENT BY 6550 IFUNNEL Q-TOF LC-MS
Pascali J.P.(j.pascali@dtolabs.eu)*, Calì A.

P3. SYNTHESIS OF THE "KROKODIL" DRUG THROUGH "STREET" REPORTED METHODS, AND PRODUCT ANALYSIS
Alves E.(manuhpa@hotmail.com)*, Soares J., Ferreira A.S., Cravo S., Neves J., Netto A., Carvalho F., Dinis--Oliveira R.

P4. DEVELOPMENT OF AN UHPLC-MS/MS METHOD FOR DETERMINING GHB AND GHB GLUCURONIDE CONCENTRATIONS IN HAIR AND APPLICATION ON FORENSIC CASES
Wang X.(xin.wang@sund.ku.dk)*, Linnet K., Johansen S.S.

P5. DETERMINATION OF NEW PYRROLIDINO CATHINONE DERIVATIVES, PVT, 4F-PVP, MPHP, PV8, PV9 AND 4F-PV9, IN HUMAN BLOOD BY MALDI-Q-TOF MASS SPECTROMETRY
Minakata K.(kminakat@hama-med.ac.jp)*, Yamagishi I., Nozawa H., Hasegawa K., Wurita A., Gonmori K., Watanabe K., Suzuki O.

P6. SIGNIFICANT INCREASE IN THE SCREENING CAPACITY OF DRUGS OF ABUSE IN ORAL FLUID THROUGH THE USE OF BIOCHIP ARRAYS
Acheson R.(scientific.publications@randox.com)*, Mullan G., Darragh J., Rodriguez M.L., Mcconnell R.I., Fitzgerald S.P.

P7. SIMULTANEOUS DETECTION AND QUANTIFICATION OF 15 DRUGS OF ABUSE AND METABOLITES IN HAIR BY ONLINE SOLID-PHASE EXTRACTION AND LC-MS/MS
Helene H., Renaud C., Deslandes G., Pineau A., Dailly E., Bouquie R., Jolliet P., Monteil--Ganiere C.(catherine.ganiere@chu-nantes.fr)*

P8. MULTIPARAMETER INVESTIGATION OF NEW PSYCHOACTIVE SUBSTANCES (NPS) BASED ON THE MATERIAL ORIGINATING FROM FORENSIC TOXICOLOGICAL PRACTICE AND FOCUSED ON METHODOLOGICAL AND MEDICO-LEGAL ASPECTS.
Klys M.(mpklys@cyf-kr.edu.pl)*, Rojek S., Maciów -- Glab M., Kula K.

P9. GENDER DIFFERENCES IN DRUG ABUSE IN THE FORENSIC TOXICOLOGICAL APPROACH.
Pieri M.(maria.pieri@unina.it)*, Buccelli C., Della Casa E.

P10. ON-SITE SCREENING OF NEW PSYCHOACTIVE SUBSTANCES BY PORTABLE MASS SPECTROMETER
Yamada M.(masuyoshi.yamada.dq@hitachi.com)*, Kaneko A., Nishimura K., Morokuma H., Kasuya F., Nakazono Y., Tsujikawa K., Iwata Y., Inoue H.

P11. REGIOISOMERIC DIFFERENTIATION OF 2-, 3-, AND 4-METHYL-ALPHA-PYRROLIDINOBUTIOPHENONE BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY USING BROMINATION
Fujii H.(fujii-hiroshi@mhlw.go.jp)*, Waters B., Hara K., Kashiwagi M., Matsusue A., Kubo S.I.

P12. DETECTION OF THE CANNABIS METABOLITE THC-COOH IN URINE AND SERUM SAMPLES BY IMMUNOASSAY: HEIA(TM) VERSUS CEDIA®
Wilhelm L.(l.wilhelm@ladr.de)*, Dormeier S., Jenckel S., Keller T.

P13. DETECTION OF BUPRENORPHINE AND NORBUPRENORPHINE IN URINE AND SERUM SAMPLES BY IMMUNOASSAY: HEIA(TM) VERSUS CEDIA®
Wilhelm L.(l.wilhelm@ladr.de)*, Dormeier S., Jenckel S., Keller T.

P14. ANALYTICAL APPROACH FOR THE IDENTIFICATION OF NEW PSYCHOACTIVE SUBSTANCES NPS IN SEIZURES AND A SNAPSHOT OF DRUGS CONFISCATED IN ITALY IN THE PERIOD 2013-2015.
Odoardi S., Romolo F.S., Strano Rossi S.(sabina.stranorossi@gmail.com)*

P15. SIMULTANEOUS DETERMINATION OF 36 NOVEL DESIGNER DRUGS OF AMPHETAMINES AND THEIR ANALOGUES IN URINE AND HAIR BY LC-MS-MS
Wang C.F., Liu Y.L.(m27030@mjib.gov.tw)*, Lee J.D., Chyueh S.D.

P16. COMPARISON OF HPLC-QTOF MS/MS ANALYSIS TO A ROUTINE EMIT, HPLC, GC/NPD AND GC/MS WORKFLOW FOR FORENSIC DRUGS OF ABUSE SCREENING
Hedman C., He X., Mcmanaway D., Pieters R., Miles A., Plath J., Taylor A.(adrian.taylor@sciex.com)*

P17. DEVELOPMENT AND VALIDATION FOR A HIGHLY SENSITIVE GC-MS/MS SCREENING AND QUANTIFICATION METHOD OF FORENSICALLY RELEVANT DRUGS OF ABUSE IN WHOLE BLOOD
Kusano M.(maiko-k@med.nagoya-u.ac.jp)*, Zaitsu K., Sakamoto Y., Miyagawa H., Tsuchihashi H., Ishii A.

P18. SENSITIVE LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY METHOD FOR THE SIMULTANEOUS DETERMINATION OF 9 KINDS OF LOCAL ANESTHETIC DRUGS
Tonooka K.(k.tonooka@ym.hamayaku.ac.jp)*, Naruki N., Honma K., Agei K., Hosono T., Kunisue Y., Terada M., Tomobe K., Shinozuka T.

P19. EVALUATION OF THE WATER FLUORIDE EXPOSURE IN URBAN AREAS IN THE PROVINCE OF BLIDA, ALGERIA
Zouani A.(aminapharmatox@hotmail.fr)*, Guerfi B., Mezroud F., Kermout I., Talailef A.

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P20. **QUANTIFICATION OF COCAINE, COCAETHYLENE AND ANHYDROECGONINE METHYL ESTER (AEME) IN WHOLE BLOOD USING HOLLOW-FIBRE LIQUID PHASE MICROEXTRACTION (HF-LPME) AND GAS-CHROMATOGRAPHY MASS-SPECTROMETRY (GC-MS)**
Sanchez C., Fonseca Pego A.M.(anamiguel14@hotmail.com)*, Yonamine M., Souza Nascimento E.

P21. **DEVELOPMENT OF THE FIRST POLYCLONAL ANTIBODY FOR THE DETECTION OF TILIDINE AND ITS ACTIVE METABOLITE NORTILIDINE**
Crawley D.(scientific.publications@randox.com)*, Vintila I., Brogan A., Benchikh M.E.O., Mcconnell R.I., Fitzgerald S.P.

P22. **EVALUATION OF A NEW ELISA KIT FOR THE SCREENING OF THE EMERGING SYNTHETIC CANNABINOID AB-PINACA IN BLOOD AND URINE**
Snelling W.(scientific.publications@randox.com)*, Darragh J., Benchikh M.E.O., Rodriguez M.L., Mcconnell R.I., Fitzgerald S.P

P23. **NEW ACQUISITION AND PROCESSING TOOLS FOR TARGETED AND UNKNOWN SCREENING APPROACHES IN TOXICOLOGY AND FORENSIC**
De Nardi C.(claudio.denardi@thermofisher.com)*, Duretz B.

P24. **SCREENING OF FASCICULOL E AND F AND ILLUDIN S IN MUSHROOMS**
Saito T.(saito@is.icc.u-tokai.ac.jp)*, Namera A., Ota S., Miyazaki S., Fujita Y., Inokuchi S.

P25. **STRATEGIES TO UNCOVER THE ADMINISTRATION OF RECOMBINANT HUMAN INSULIN BY LC-HRMS**
Thomas A.(a.thomas@biochem.dshs-koeln.de)*, Schänzer W., Thevis M.

P26. **ESTIMATION OF THE STRUCTURE OF SYNTHETIC CANNABINOIDS USING GC–EI–MS AND GC–PCI–MS**
Saito T.(saito@is.icc.u-tokai.ac.jp)*, Namera A., Nakamoto A., Kawamura M., Inokuchi S.

P27. **SIMULTANEOUS DETERMINATION OF MUSHROOM TOXINS α-AMANITIN, ß-AMANITIN AND MUSCARINE IN HUMAN URINE BY SOLID-PHASE EXTRACTION AND ULTRA-HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED WITH ULTRA-HIGH-RESOLUTION TOF MASS SPECTROMETRY**
Tomková J.(jantomkova@gmail.com)*, Ondra P., Válka I.

P28. **DERIVATIZATION OF 15 CATHINONE ANALOGS WITH 2,2,2-TRICHLOROETHYL CHLOROFORMATE - A GAS CHROMATOGRAPHY - MASS SPECTROMETRY STUDY**
Frison G.(giampietro.frison@ulss12.ve.it)*, Frasson S., Zamengo L., Bettin C., Zancanaro F., Sciarrone R.

P29. **ETHANOL WORKBOOK (ETWB): A USER-FRIENDLY SOFTWARE APPLICATION FOR MEASUREMENT UNCERTAINTY CALCULATION, DATA MANAGEMENT, COMPLIANCE ASSESSMENT AND QUALITY CONTROL OF FORENSIC BLOOD ALCOHOL CONTENT DETERMINATIONS**
Zamengo L.(luca.zamengo@ulss12.ve.it)*, Frison G., Frasson S., Sciarrone R.

P30. **RAPID SCREENING METHOD COVERS COMMON 161 FORENSIC DRUGS BY ULTRA-HIGH SPEED LC/MS/MS WITH SYNCHRONIZED SURVEY SCANNING**
Minohata T.(minohata@shimadzu.co.jp)*, Kudo K., Shima N., Katagi M., Usui K., Tsuchihashi H., Ikeda N.

P31. **THE USE OF IONIC LIQUIDS FOR THE FAST AND SIMPLE EXTRACTION OF BENZODIAZEPINES FROM COMPLEX BIOLOGICAL SAMPLES**
De Boeck M.(marieke.deboeck@kuleuven.be)*, Dehaen W., Tytgat J., Cuypers E.

P32. **MULTIVARIATE STATISTICAL EVALUATION OF FATTY ACID ETHYL ESTERS (FAEES) COMBINED WITH OTHER BIOLOGICAL MARKERS FOR DIAGNOSIS OF ALCOHOL ABUSE**
Gerace E., Giacomelli L., Alladio E., Salomone A.(alberto.salomone@antidoping.piemonte.it)*, Vincenti M.

P33. **ANALYSIS OF ALKALOIDS OF CHELIDONIUM MAJUS BY THERMAL DESORPTION SURFACE-IONIZATION SPECTROSCOPY METHOD**
Zulfikariyeva D.A., Yuldashev Z.A. (z.yuldashev65@gmail.com)*, Ibragimova M.M.

P34. **DETERMINATION OF TETRAHYDROCANNABINOL (THC) AND ITS MAIN METABOLITES USING GC TRPLE QUADRUPOLE.**
Albertini T.(tommaso.albertini@thermofisher.com)*

P35. **APPLICATION OF UV-SPECTROPHOTOMETRY METHOD IN THE ANALYSIS OF PIROXICAM ISOLATED FROM BIOLOGICAL OBJECT**
Yuldashev Z.A. (z.yuldashev65@gmail.com)*, Najimitdinova N., Ibragimova M.M.

P36. **USE OF THE HPLC METHOD IN THE ANALYSIS OF PIROXICAM, ISOLATED FROM BIOLOGICAL LIQUIDS**
Yuldashev Z.A. (z.yuldashev65@gmail.com)*, Najimitdinova N., Ibragimova M.M.

P37. **CHEMICAL CHARACTERIZATION AND MICROBIOLOGICAL ANALYSIS FOR QUALITY CONTROL OF METFORMIN DRUG SUBSTANCE**
Guerfi B.(bahdja.guerfi@gmail.com)*, Zouani A., Hamidi S., Hamidi S., Hadjadj Aoul F.Z.

P38. **AN AUTOMATED UHPLC-MS/MS METHOD FOR IDENTIFICATION AND QUANTIFICATION OF 10 BENZODIAZEPINES IN ENZYMATICALLY HYDROLYZED DILUTED URINE.**
Hedman E.(erik.s.hedman@karolinska.se)*, Beck O.

P39. **A NOVEL AND FAST WORKFLOW FOR FORENSIC TOXICOLOGICAL SCREENING AND QUANTITATION USING QTOF LC-MS/MS SYSTEM**
He Kevin X., Taylor A.(adrian.taylor@sciex.com)*, Jarvis M., Cox D., Wang A.

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P40. THE DEVELOPMENT OF AN LC-MS/MS SCREENING METHOD FOR 104 TARGETED COMPOUNDS IN WHOLE BLOOD, USING LIBRARY SEARCHING ON A QTRAP MASS SPECTROMETER
Singletary H., Jarvis M., Taylor A.(adrian.taylor@sciex.com)*

P41. THE NEVER ENDING STORY OF CANNABINOIDS IN HAIR
Salomone A.(alberto.salomone@antidoping.piemonte.it)*, Seganti F., Gerace E., Di Corcia D., Vincenti M.

P42. HIGH SENSITIVITY ANALYSIS OF THC-COOH IN HAIR BY USING AN UFMS GCMS TQ IN NEGATIVE CHEMICAL IONIZATION MODE
Scollo G., Schulte H.(hs@shimadzu.eu)*, Moreau S.

P43. FULLY AUTOMATED GC/MS DETERMINATION OF THC, CBN AND CBD IN HAIR BY GC/MS
Lerch O.(oliver_lerch@gerstel.de)*, Heinl S., Erdmann F.

P44. DEVELOPMENT, OPTIMIZATION AND VALIDATION OF AN ANALYTICAL METHOD FOR THE ANALYSIS OF DRUGS BY GC-MS IN POST MORTEM LIVER SAMPLES USING QUECHERS FOR SAMPLE PREPARATION
Pereira D., Costa S., Barroso M., Fonseca S.(sfonseca@dlinml.mj.pt)*, Dias M., Franco J.M., Borges C.M., Castañera A.

P45. COMBINING COMPLIMENTARY ION RATIO AND LIBRARY MATCHING CONFIRMATORY TECHNIQUES IN ONE LC-MS/MS METHOD
Mcclure E., Jarvis M., Taylor A.(adrian.taylor@sciex.com)*

P46. RAPID SCREENING OF 56 DRUGS OF ABUSE WITH ACCURATE MASS FRAGMENT LIBRARY USING DIRECT ANALYSIS IN REAL TIME (DART) COUPLED TO TIME-OF-FLIGHT MASS SPECTROMETRY (TOF-MS)
Zhang Y., Lian R., Wu Z., Yan S., Lv X., Yuan X., Wang R., Liang C., Ni C., Wang W.(yr_zhang@126.com)*

P47. THE DEVELOPMENT OF NEW PSYCHOACTIVE SUBSTANCES IN FRANCE
Roussel O.(queneu94@gmail.com)*, Carlin M., Tensorer L., Bouvot X., Balter C., Sabini S.

P48. DEVELOPMENT AND VALIDATION OF A METHOD FOR THE DETERMINATION OF SELECTED OPIATES IN WHOLE BLOOD USING MICROEXTRACTION IN PACKED SORBENT
Figueirinha D., Oppolzer D., Restolho J.(j.restolho@nal-vonminden.com)*, Barroso M., Gallardo E.

P49. DEVELOPMENT OF THE FIRST POLYCLONAL ANTIBODY FOR THE DETECTION OF THE SYNTHETIC OPIOID AH-7921 AND ITS MAIN METABOLITE NOR-AH-7921
Crawley D.(scientific.publications@randox.com)*, Vintila I., Taggart J., Benchikh M.E.O., Rodriguez M.L., Mcconnell R.I., Fitzgerald S.P.

P50. FLUORIDE REGENERATION AND GC-MS WITH LARGE VOLUME INJECTION OF O-ALKYL-METHYLPHOSPHONOFLUORIDATES FOR VERIFYING NERVE GAS EXPOSURE
Seto Y.(seto@nrips.go.jp)*, Kanamori--Kataoka M., Nagoya T., Tsuge K., Ohmori T., Sasano R., Matsuo S., Uchida S.

P51. A RAPID HEAD SPACE-GAS CHROMATOGRAPHY MASS SPECTROMETRY METHOD FOR DETERMINATION OF CYANIDE IN BLOOD SAMPLES
Destanoglu O.(destanoglu@yandex.com)*, Yeter O., Korkut S., Ates I.

P52. GAS CHROMATOGRAPHY/MASS SPECTROMETRY METHOD FOR THE SIMULTANEOUS DETERMINATION OF 9 LOCAL ANESTHETIC DRUGS AND ITS INTERNAL STANDARD (LIDOCAINE-D10) IN HUMAN WHOLE BLOOD, URINE AND SPUTUM
Kunisue Y.(kunisue@mst.or.jp)*, Sasaki Y., Yoshizawa C., Tonooka K., Shinozuka T.

P53. LC-MS/MS SCREENING OF ILLICIT AND MEDICINAL DRUGS ON DRIED BLOOD SPOTS
Xiang P.(xiangping2630@163.com)*, Gerostamoulos D., Drummer O.H.

P54. A COMPREHENSIVE SCREENING APPROACH TO DETECT TOXICOLOGICAL COMPOUNDS IN HUMAN MATRICES IN FORENSIC CASES.
Verschraagen M., Gonsalves J.(j.gonsalves@nfi.minvenj.nl)*, Lusthof K.

P55. SCREENING FOR CHEMICAL (WARFARE) AGENTS IN HUMAN MATRICES
Van Der Hulst R.(r.van.der.Hulst@nfi.minvenj.nl)*, Boone C., Drouin L., Verschraagen M.

P56. DEVELOPMENT OF MULTI-TARGETED SCREENING METHOD FOR 56 NATURAL TOXINS IN PLASMA BY LC/Q-TOFMS
Ogawa T.(ogawatd@aichi-med-u.ac.jp)*, Zaitsu K., Shiraishi Y., Kusano M., Iwai M., Tsuchihashi H., Ishii A., Seno H.

P57. INFLUENCE OF THE SAMPLE PREPARATION FOR THE DETERMINATION OF THC AND THE METABOLITES THC-OH AND THC-COOH IN HAIR SAMPLES
Franz T.(t.franz@ftc-munich.de)*, Dame T., Sachs H., Schwarz G., Musshoff F.

P58. BEHAVIOR OF HYGRINE AND CUSCOHYGRINE IN ILLICIT COCAINE PRODUCTION ESTABLISHES THEIR USE AS MARKERS FOR DISCRIMINATION BETWEEN CHEWING OF COCA LEAVES AND COCAINE ABUSE
Rubio N.C.(cristinarubio2@gmail.com)*, Hastedt M., Krumbiegel F., Pragst F.

P59. MEASUREMENT UNCERTAINTY IN DETERMINATION OF AMPHETAMINES IN URINE BY LIQUID-PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY
Carobini Werner De Souza Eller Franco De Oliveira S.(sarahcarobini@usp.br)*, Yonamine M.

P60. METHODS FOR THE DETECTION OF FETAL ALCOHOL EXPOSURE
Santos F.S.D.(fabispineti@yahoo.com.br)*, De Martinis B.S., Furtado E.F.

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P61. **DETERMINATION OF BARBITURATES IN HAIR MATRIX BY LIQUID PHASE MICROEXTRACTION (LPME) AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)**
Lopes Roveri F.(flavia_rov@hotmail.com)*, Passos Bismara Paranhos B.A., Yonamine M.

P62. **COLLISION-INDUCED DISSOCIATION PATHWAYS OF HALLUCINOGENIC PHENETHYLAMINES (2C-X) AND THEIR N-(2-METHOXYBENZYL) DERIVATIVES (NBOME) USING HIGH-RESOLUTION MASS SPECTROMETRY FOR NON-TARGETED SCREENING PURPOSES**
Pasin D.(daniel.pasin@student.uts.edu.au)*, Fu S., Cawley A.

P63. **GAS CHROMATOGRAPHY – TRIPLE QUADRUPOLE MASS SPECTROMETRY DETERMINATION OF SILDENAFIL AND ITS ANALOGUES IN DIETARY SUPPLEMENTS**
Mokhtar Siti U.(siti.mokhtar@monash.edu)*, Chin S., Kee C., Low M., Drummer O.H., Marriott Philip J.

P64. **CHARACTERIZATION AND IN-VITRO PHASE I METABOLITE IDENTIFICATION OF THE DESIGNER BENZODIAZEPINES CLONAZOLAM, DESCHLOROETIZOLAM, AND MECLONAZEPAM**
Huppertz L.(laura.huppertz@uniklinik-freiburg.de)*, Bisel P., Westphal F., Franz F., Auwärter V., Moosmann B.

P65. **VALIDATION OF A NEWLY DEVELOPED IMMUNOLOGICAL LATERAL FLOW RAPID TEST FOR FAST AND EFFICIENT SOLID SUBSTANCE TESTING (TRACEDETECT)**
Winkler T., Bernhard W.(wernerbernhard@msn.com)*, Zander T.

P66. **EFFECT OF METHANOL AS A SOLVENT FOR COCAINE USED AS A REFERENCE MATERIAL**
Da Silva Lima Oliveira C.(celinalvasloliveira@gmail.com)*, Fernandes Araujo Carvalho M., Leal Cunha R.

P67. **EVALUATION OF DRUGS OF ABUSE EXTRACTION FROM ORAL FLUID USING SUPPORTED LIQUID EXTRACTION (SLE) PRIOR TO UPLC/MS-MS ANALYSIS**
Williams L., Jones R.(rhys.jones@biotage.com)*, Lodder H., Senior A., Edgington A., Davies G., Jordan S., Desbrow C.

P68. **EXTRACTION OF PROPOFOL FROM WHOLE BLOOD USING SUPPORTED LIQUID EXTRACTION (SLE) PRIOR TO GC/MS ANALYSIS**
Jones R.(rhys.jones@biotage.com)*, Williams L., Senior A., Edgington A., Davies G., Lodder H., Jordan S., Desbrow C.

P69. **EXTRACTION OF DELTA-9-THC, THCA AND 11-NOR-9-CARBOXY-THC FROM ORAL FLUID USING SUPPORTED LIQUID EXTRACTION (SLE) AFTER COLLECTION WITH THE QUANTISAL, INTERCEPT & ORAL-EZE COLLECTION DEVICES PRIOR TO GC/MS ANALYSIS**
Jones R.(rhys.jones@biotage.com)*, Williams L., Senior A., Edgington A., Davies G., Lodder H., Jordan S., Desbrow C.

P70. **EXTRACTION OF DELTA-9-THC, 11-HYDROXY-DELTA-9-THC AND 11-NOR-9-CARBOXY-THC FROM WHOLE BLOOD AND 11-NOR-9-CARBOXY-THC FROM URINE USING SUPPORTED LIQUID EXTRACTION (SLE) PRIOR TO GC/MS ANALYSIS**
Jones R.(rhys.jones@biotage.com)*, Teehan K., Williams L., Senior A., Edgington A., Davies G., Lodder H., Jordan S., Desbrow C.

P71. **SUPERCRITICAL FLUID CHROMATOGRAPHY (SFC) AS ORTHOGONAL TECHNIQUE FOR IMPROVED DETECTION OF POLAR ANALYTES IN ANTI-DOPING CONTROL**
Parr M.K.(maria.parr@fu-berlin.de)*, Wüst B., Nägele E., Botre F.

P72. **IDENTIFICATION OF 1,3-DIMETHYLAMYLAMINE (1,3-DMAA) IN FOOD SUPPLEMENTS USING DIFFERENT DERIVATIZATION REACTIONS AND ANALYSIS BY GC-MS: A CASE REPORT**
Da Silva Lima Oliveira C.(celinalvasloliveira@gmail.com)*, Maria Portela De Santana M., Leal Cunha R.

P73. **UTILITY AND APPLICATION OF DNA-BASED FORENSIC INVESTIGATIONS IN DOPING CONTROL**
Stampella A.(ale.stampella@gmail.com)*, Botrè F., De La Torre X., Pirri D., Donati F.

P74. **GAS PHASE ION SEPARATION OF DRUGS USING A MODIFIER-ASSISTED ELECTROSPRAY IONIZATION-DIFFERENTIAL MOBILITY-MASS SPECTROMETER SYSTEM**
Chen P.S.(paishanchen@ntu.edu.tw)*

P75. **DEVELOPMENT AND VALIDATION OF TARGETED DRUG SCREENING METHOD FOR BLOOD SAMPLES BY LIQUID CHROMATOGRAPHY-TIME OF FLIGHT/ MASS SPECTROMETRY**
Pantatan S.(s_pantatan@yahoo.com)*, Limcharoen S., Kantajai S., Chaiya S., Leelapojanaporn A.(leeampika@yahoo.com)*

P76. **STUDIES OF THE VOLATILITY OF AMPHETAMINE AND ITS DERIVATIVES METHAMPHETAMINE, MDA, MDMA, AND MDEA BY VACUUM CENTRIFUGATION AND GC-MS ANALYSIS**
Meyer G.M.(golomagnus.meyer@kssg.ch)*, Val M., Beyer J.

P77. **DEVELOPMENT OF A MULTI-PARAMETER ANALYTICAL APPROACH FOR THE DETECTION OF "INDUCED HYPOXIA" IN BLOOD DOPING**
Pirri D.(danipirri88@hotmail.it)*, De La Torre X., Stampella A., Botre F., Donati F.

P78. **RECENT ADVANCES ON THE APPLICATION OF GC-IRMS IN ANTI-DOPING ANALYSIS: STABILITY AND UTILITY OF LONGITUDINAL DATA**
Curcio D.(d.curcio30@gmail.com)*, Botre F., Colamonici C., Molaioni F., Procida G., De La Torre X.

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Lectures

Opening Lecture by Marilyn Huestis, USA

A Conversation with Lucrezia Borgia: Saint or Sinner?

Marilyn A. Huestis, Chief, Chemistry & Drug Metabolism, IRP, National Institute on Drug Abuse, National Institutes of Health

One of the most infamous Italians, Lucrezia Borgia, was born April 18, 1480, Subiaco, Italy to Cardinal Rodrigo Borgia, who became Pope Alexander VI & his mistress, Vannozza dei Cattanei. She had four brothers, Juan, the evil Cesare, Giovanni and Gioffre Borgia. Lucrezia Borgia was an Italian noblewoman who was inextricably, and perhaps unfairly, linked to the crimes and debauchery of her family. It was said that she had incestuous affairs with her father and her brother, Cesare, and bore a son, perhaps by one of them. The family was known to be expert at the black art of poisoning, one of the many arts cultivated in Renaissance Italy. Lucrezia, the infamous Black Widow, was said to murder with her poison ting, a ring drenched in arsenic and a sharp needle to get the poison into the blood. She was a golden-haired beauty, whose crimes and arranged marriages advanced the power and wealth of the Borgia family. At 11, she was betrothed by her father to a man she hadn't met, but before the wedding the Borgia's fortunes changed, as her father became Pope Alexander VI in 1492. He wanted to control all Italian principalities, and marrying Lucrezia to powerful nobility was one tool to achieve the desired consolidation. Marriages were arranged when she was 13 to Giovanni Sforza, Lord of Pesaro and Count of Catignola to establish an alliance with the powerful Milanese Ducal family. When this union was no longer needed, the Pope forced Giovanni to declare that he was impotent and the marriage was annulled. However, when she emerged from a convent after the annulment, she was pregnant with someone's child. Two papal decrees claimed Giovanni's father was her brother Cesare, and later, her father the Pope. At the age of 18, she wed Alfonso of Aragon, the handsome 17-year-old Duke of Bisceglie and son of the late king of Naples. They were very happy and had a child together, but her brother Cesare murdered him as he left the papal chambers. Finally, she was betrothed to Alfonso I d'Este, Duke of Ferrrara, in 1502. He was reluctant due to Lucrezia's terrible reputation for incest and murders by poisonings, termed dinner-party executions. It was said that those invited to dine at Lucrezia's table made out their wills before they left home. She favored a poison called Cantarella, a variation of arsenic, often used for political assassinations, highly effective and difficult to trace. This poison also contained phosphorus, a secret divulged by a Spanish monk. She also purportedly used a deadly concoction called Monkshood, Wolfbane or Veninum Lupinum, composed of aconite, taxus baccata, caustic lime, arsenic, bitter almonds, powdered glass and honey, and shaped into walnut-sized pills. Other favorites were foxglove or digitalis that interferes with the sodium-potassium pump, producing ventricular tachycardia, and henbane, Hyoscyamus niger, that is poisonous and psychoactive. In Shakespeare's "Hamlet," Claudius kills Hamlet's father by pouring a tincture of "cursed hebenon" into his ear. Despite evidence of her guilt, it is unclear whether Lucrezia was a pawn in the wicked games of her family, used by her father and brother to advance their own political agendas, or whether she really was the ultimate "Black Widow." Lucrezia Borgia died at the age of 39 during the birth of her 8th child. Memorialized in paintings, poems and even an opera- was she a saint or a sinner- what do you think?

Professor Dr. Dr. (h.c.) Marilyn A. Huestis is a tenured senior investigator and Chief, Chemistry and Drug Metabolism Section, IRP, National Institute on Drug Abuse, National Institutes of Health and Adjunct Professor in the Department of Epidemiology and Preventive Medicine, School of Medicine, University of Maryland. She thoroughly enjoys mentoring doctoral students in Toxicology, has to date directly overseen the research of 16 distinguished new toxicologists, and currently has 2 students pursuing their dissertation research. Her research program seeks to discover mechanisms of action of cannabinoid agonists and antagonists, effects of in utero drug exposure, and the neurobiology and pharmacokinetics of novel psychoactive substances, the emerging face of drug abuse. She has published 381 peer-reviewed manuscripts and book chapters and more than 500 abstracts at national and international meetings. Professor Huestis received a bachelor's degree in biochemistry from Mount Holyoke College (cum laude), a master's degree in clinical chemistry from the University of New Mexico (with honors), and a doctoral degree in toxicology from the University of Maryland (with honors). Professor Huestis received a Doctor Honoris Causa from the Faculty of Medicine, University of Helsinki in Finland in 2010. Other important awards include, Distinguished Fellow Award from the American Academy of Forensic Sciences (AAFS) in 2015, The International Association of Forensic Toxicologists (TIAFT) Alan Curry Award in 2010, the American Association for Clinical Chemistry Outstanding Contributions in a Selected Area of Research Award in 2008, the International Association of Therapeutic Drug Monitoring and Clinical Toxicology (IATDMCT) Irving Sunshine Award in 2007, the AAFS Rolla N. Harger Award in 2005, and the Irving Sunshine Award for Outstanding Research in Forensic Toxicology in 1992. The journal Clinical Chemistry featured her as an "Inspiring Mind". She currently serves on the new National Commission on Forensic Sciences, and the Organization of Scientific Area Committee on Toxicology, World Anti-doping Agency's Prohibited List Committee, the Scientific Working Group on Toxicology (SWG-TOX), Transportation Research Board Committee on Alcohol and Other Drugs, and the National Safety Council's Alcohol, Drugs and Impairment Division Executive Board. Professor Huestis is past president of the Society of Forensic Toxicologists, past Chair of the Toxicology Section of the American Academy of Forensic Sciences, and the first woman president of The International Association of Forensic Toxicologists.

Lectures

First Lecture by Giovanni Serpelloni, ITALY

Addiction Neuroscience and Toxicology: a New Opportunity

Research Senior Consultant, University of Florence

The discipline of forensic toxicology has developed greatly in recent years. Various disciplines like medicine, biology and chemistry have mutually contributed to its growth, in addition to the work and genius of researchers. The toxic effects of substance abuse, their metabolism, the best way to discover and classify them, their consequences have been well-rooted by forensic science and continue to be the subject of major and fundamental research and discoveries. Simultaneously, addiction neuroscience notably functional neuroimaging studies of the effects of substances and both structures (micro and macro) of the brain as well as on its cognitive functions, have been enhanced thanks to the development and availability of new and advanced technologies.
The idea to combine and integrate the models, paradigms and methodologies of investigation in addiction neuroscience and forensic toxicology, in recent years, has prompted many knowledgeable researchers to talk to each other.
We believe that the two sets of knowledge, if well integrated and finalized, can generate new models of interpretation of the toxicological complex reality related to the use of drugs.
The meeting of scientists who study the brain, the neurobiological working mechanisms along with neuro-psychological aspects of addiction, and forensic toxicologists, can only be the bearers of innovation and good prospects for both disciplines.
This meeting would also serve as a more solid foundation and stricter scientifically rational aspects related to the interpretation of violent behavior and / or anti-social and criminal behavior, in relation to substance use. We believe it is important and very useful for forensic toxicologists, for example, to know the neuropsychological mechanisms of how substances can affect the proper functioning of the prefrontal cortex and its role in the voluntary control of behavior, which are the pathological conditions that can change this important function, as and when these substances can influence the expression of epigenetic trim and thus the expression of brain receptors of the dopaminergic system or as the endocannabinoid system (and therefore some higher cognitive functions and consequently the behavior) can vary under the influence of substances. Moreover, the substances may compromise the neurobiological and cognitive phenomena of the storage and create, in the hippocampus of the person, perceptual distortions and "false memories", thus invalidating the potential to remember correctly and therefore to be reliable witnesses.
Interpreting the use of substances and the behaviors of the person in the forensic context, through this dual, integrated and simultaneous observation, it is definitely innovative and very exciting. The reflection that we would like to propose then is to promote and encourage an increasingly integrated & balanced approach between the two disciplines. All of this seen through different lenses where on one side there we find forensic toxicology and on the other side addiction neuroscience, with an open mind and serene look to the future, perceiving this proposal not as a threat or a kind of "pollution" but a new perspective, a new opportunity for all researchers. A proposal that could possibly also be useful for society to create a better justice based increasingly on scientific evidence in order to properly interpret the relationship between the presence of substances - neuropsychological effects and the expression of possible criminal behavior .

A medical doctor with over 30 years of experience, he has dedicated his work to the study and analysis of the neuroscience of addiction, in clinical and research settings. He has directed and funded the most important Italian national project on neuroscience of addiction and neuroimmaging, toxicology research and neurobiology.
He has been instrumental in collaborating with research institutes from the US government (NIH.NIDA - National Institute on Drug Abuse - Bethesda, USA), with which he created and signed agreements regarding the development of common projects and interactions in the area of research, treatment, prevention and rehabilitation of addictions (at the White House in Washington D.C. in 2011).
He has covered high institutional positions for the Italian Government's National Public Health System as Head of the Department of Drug Control Policy (DPA) of the Presidency of the Council of Ministers, National Coordinator of Drugs in Europe (EU Council) on behalf of the Italian Presidency of the Council of Ministers, and member of the Horizontal Drugs Group (HDG) of the Council of Europe - Brussels.
In addition, he was on the Board of Directors of the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) as full Italian Representative, and Representative of the delegation of the Italian Government to the Commission on Narcotic Drugs of the United Nations (Vienna).
He founded and directed the National School on Addiction at the School of Public Administration of the Presidency of the Council of Ministers, and is Director in charge of the "Italian Journal on Addiction", in collaboration with the United Nations (UNICRI).
He founded and directed the National Early Warning System (NEWS) on Drugs.
He was also appointed Honorary Judge of the US Courts on drugs (Special Courts) and collaborated with United Nations Interegional Crime and Justice Research Institute (UNICRI – Vienna) and with United Nations Office on Drugs and Crime (UNODC – Vienna).
He is author of over 200 scientific publications, member of the Italian Association "Group of Italian Forensic Toxicologists- GTFI", Addiction Neuroscience and Forensic Toxicology Senior Consultant, and professor lecturer of PFPC Master at the University of Florence.

Lectures

Closing Lecture by Steven B. Karch, USA

The Story of Hurricane Katrina

Consultant Pathologist/Toxicologist
Berkeley, California

Hurricane Katrina was one of, if not, the greatest natural disaster ever to strike the United States. By the evening of Monday, August 29, 2005, more than 80 percent of New Orleans lay underwater. By the time the water had receded and a body count attempted, more than 1,600 lives had been lost, 81,000 businesses had been damaged or destroyed, and more than 200,000 homes had been washed away. Three days after the storm, 42 decomposing bodies were found in the hulk that had been Memorial Hospital. The misery of the conditions under which they died can never truly be described. Memorial Hospital was without electricity, had no water, no air conditioning, inadequate supplies, a non-functioning ventilation system, and the temperature inside remained at a relatively constant 110°F (43°C) Toilets were overflowing, bodies were decomposing, and the stench was overbearing. No one can pass judgment on what happened at Memorial unless they were there. One doctor, assisted by two nurses stayed for the duration. For her efforts she was charged with 10 counts of homicide, all counts based upon drug concentrations measured in decomposed corpses. The District Attorney (Solicitor General), who was running for reelection, personally announced the charges. He lost the election and she was ultimately acquitted of all charges. If it were possible to identify just one single feature that made Hurricane Katrina so devastating, most agree it was the complete and utter failure of the local, state, and federal governments to offer aid to the distressed and dying. The horrible irony is that the United States very likely has the world's most sophisticated system for identifying victims of mass disasters, called DMORT. This talk will graphically illustrate the problems faced by local officials; explain the workings of DMORT, and explore the interface between forensic science and the media.

Dr. Karch received his undergraduate degree in Philosophy from Brown University, attended graduate school in Anatomy and Cell Biology at Stanford University, and received his MD from Tulane University School of Medicine in New Orleans. He did postgraduate training in neuropathology at the Royal London Hospital, and in Cardiac Pathology at Stanford University, Palo Alto. He served as consultant cardiac pathologist at the Office of the San Francisco Medical Examiner for nearly 10 years. In 2006 he was elected a fellow of the Royal College of Physicians, Faculty of Forensic and Legal Medicine, and in 2007 he became the first American ever elected to the Italian Academy of Forensic Toxicology.He has written more than 150 peer-reviewed papers and book chapters, most having to do with the investigation of drug-related deaths. He has published more than a dozen books. The 5th edition of his textbook, Karch's Pathology of Drug Abuse is to be released within a few weeks. It is a book used by medical examiners around the world. Dr. Karch serves on the editorial board of the Journal of Cardiovascular Toxicology, the Minerva Medico Legale (Rome), and Medicine, Science and Law (London). Dr. Karch is a fellow and active member of the American Academy of Forensic Sciences (AAFS), the Society of Forensic Toxicologists (SOFT), the National Association of Medical Examiners (NAME), and the Royal Society of Medicine (RSM) in London, the International Association of Forensic Toxicologists (TIAFT) and the Italian Association "Group of Forensic Toxicologists" (GTFI). Dr. Karch tries to limit has cases to those involving drug-related death and has spent much of the last 10 years as a consultant to the State of Louisiana investigating the deaths that followed Hurricane Katrina. He is generally considered a leader in the field of drug death investigation. He has lived with his wife Donna in Berkeley, California, for the last 40 years. He remains in active practice and is currently writing a history of Napoleon and his doctors

Abstracts

53rd TIAFT meeting 2015
FIRENZE
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9.30-11.00am SESSION 1 – Toxicokinetics I

MOP 1 TOXICOKINETICS OF NEW PSYCHOACTIVE SUBSTANCES: DETERMINATION OF THEIR PLASMA PROTEIN BINDING AND ITS USE IN CALCULATING METABOLIC STABILITY

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Only free drugs can cause pharmacological effects and can be metabolized and excreted. Hence, their binding to plasma proteins is an important parameter, which is usually studied for therapeutic drugs, but not for drugs of abuse in particular new psychoactive substances (NPS). However, plasma protein binding can be important for predicting drug-drug interaction and estimating metabolic stability of drugs.
Representative of different NPS classes should be tested for their amount of plasma protein binding. Protein binding data should then be used to calculate their so-called metabolic stability after pooled human liver microsomes (pHLM) incubations. Finally, results should be compared to published in vivo data.
The amount of plasma protein binding was determined after ultrafiltration using Centrifree devices (Millipore) and LC-MS (AT LC-MSD). The metabolic stability was calculated as hepatic first-pass extraction using the intrinsic clearance (Clint,app, mL/min/kg) after pHLM incubations (0-30 min) designed as parent compound disappearance assay. Incubations (0-30 min) were done at two different substrate concentrations (2.5 and 25 µM) and analyzed by LC-MSn (TF LXQ).
Plasma protein binding was determined for 16 NPS representing four different classes (cathinones, synthetic cannabinoids, herbal drugs, and miscellaneous). Values reached from <60% for cathinones to >99% for synthetic cannabinoids. Calculated intrinsic clearance for WIN 55,212-2 was between 16 – 20 mL/min/kg indicating a high hepatic first-pass extraction similar to already published metabolism data.
A strategy for determination of protein binding for NPS was established successfully and its application for estimating their metabolic stability shown.

MOP 2 CROCODILE: METABOLISM OF DESOMORPHINE IN HUMAN LIVER MICROSOMES, HUMAN HEPATOCYTE CULTURES, AND RAT URINE AS WELL AS ITS DETECTABILITY USING OUR STANDARD URINE SCREENING APPROACHES

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Desomorphine (DM, Krokodil, Crocodile, Krok, Russian Magic), an old therapeutic drug, appeared on the illicit drug market in the last years as a homemade product and a substitute for heroin. Chemistry, synthesis, analytics, prevalence, use, pharmacology, and toxicology of Krokodil were recently reviewed by Katselou et al., Life Sci, 2014. The aim of the present study was to investigate the in vitro and in vivo metabolism of DM as well as its detectability using GC-MS, LC-high resolution (HR)-MS/MS, and LC-MSn techniques.
For in vitro studies, DM (25 µM) was incubated with pooled human liver microsomes (pHLM) according to Welter J et al., ABC, 2014. Reactions were stopped with ice-cold acetonitrile, the samples centrifuged, and the supernatants analyzed by LC-HR-MS/MS (TF Q-Exactive). Furthermore, 2D monolayer and 3D spheroid cultures HepaRG and HepG2 cells were exposed to DM (10µM) for 24 h according to Mueller D et al. Toxicol In Vitro, 2014. For in vivo studies, 20 or 1 mg/kg body mass of DM were administered to male Wistar rats for toxicologic diagnostic reasons. For the identification of the phase I and II metabolites, urine samples after high dose were analyzed by GC-EI-MS (AT GC-MSD) and/or LC-HR-MS/MS according to Helfer AG et al., DTA, 2014. For SUSAs, the rat urine samples after low dose were analyzed either after acidic hydrolysis, liquid-liquid extraction, and acetylation by full-scan GC-MS or after precipitation by LC-MSn (TF LXQ) according to Helfer AG, DTA, 2014.
Besides DM, the following metabolites could be identified in the given incubation assays: pHLM: nor-DM, hydroxy-DM, and DM-N-oxide; 2D HepaRG: nor-DM, hydroxy-DM, DM-N-oxide, DM glucuronide, nor-DM glucuronide, DM-N-oxide glucuronide, and DM sulfate; 2D HepG2: nor-DM, hydroxy-DM, DM-N-oxide, DM glucuronide, nor-DM glucuronide, and DM sulfate; 3D HepaRG: nor-DM, hydroxy-DM, DM-N-oxide, DM glucuronide, nor-DM glucuronide, and DM-N-oxide glucuronide; 3D HepG2: nor-DM, hydroxy DM, DM-N-oxide, and DM glucuronide. In rat urine (high dose), DM, nor-DM, hydroxy-DM, DM-N-oxide, DM glucuronide, nor-DM glucuronide, hydroxy-DM glucuronide, DM-N-oxide glucuronide, and DM sulfate could be detected. In rat urine (low dose), desomorphine and its main metabolites could be detected using the GC-MS and LC-MSn SUSAs.
Desomorphine and its phase I metabolites were identified in pHLM, the four hepatocyte incubations, and rat urine while the phase II metabolites, as expected, only in the hepatocytes and rat urine. Further studies are in progress to assess the most suitable human cell model for the prediction of human hepatic metabolism. Intake of the heroin substitute DM should also be detectable in human urine by SUSAs assuming corresponding doses and similar pharmacokinetics in rats and humans.

MOP 3 **STUDIES ON THE STABILITY OF COCAINE, MDMA, MDEA, AND THEIR MAIN HUMAN URINARY METABOLITES IN WASTEWATER BY HILIC LC-MS/MS, USING POOLED HUMAN URINE AND URINATED SOIL SAMPLES**

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Currently, the main targets for monitoring consumption of drugs of abuse in wastewater are unchanged parent compounds rather than their urinary metabolites. This does not allow discriminating between consumption and disposal into the sewer system. Analyzing their urinary metabolites could overcome this issue. However, data is needed on metabolite stability in wastewater

The aim of the study was to develop an analytical method based on HILIC LC-MS/MS to monitor the stability of cocaine, MDMA and MDEA, and their main human urinary metabolites in wastewater. The method should then be applied to analyze urine and urinated soil samples incubated in wastewater to study stability. Additionally, alterative targets for MDMA in wastewater analysis should be proposed.

Cocaine, MDMA, and MDEA and seven of their main human metabolites were analyzed using a LUNA HILIC analytical column and an Agilent 6410 MS/MS with positive ESI. Validation of the method was based on European Medicines Agency guidelines for bioanalytical method validation.Pooled human urine and urinated soil samples were diluted with methanol, centrifuged, and evaporated under nitrogen to concentrate the analytes. The concentrated samples were then diluted with fresh wastewater or miliQ water, and incubated at 23 °C or 5 °C for 24h. Samples were taken at different time points for analysis. Sample preparation of the wastewater incubations consisted of mixing with methanol and filtration. Finally, a 2 µL aliquot was injected onto the LC-MS/MS for analysis. A compound was found stable, when ± 15 % remained compared to the beginning of the incubation.

Benzoylecgonine decreased to 79 ± 10% after 24 hours. Ecgonine methyl ester and trace amounts of cocaine rapidly decreased within the first 4 h. For cocaethylene, 51 ± 18% remained after 24h but it could be shown to be stable up to 8h. 4-hydroxy-3-methoxymetamphetamine (HMMA) was stable for up to 8h (100 ± 10%) and there was an overall increase in its concentration after 24h (129 ± 42%), this was possibly due to microbial transformation of MDMA and cleavage of HMMA glucuronide during incubation. HMMA- and 3,4-dihydroxymethamphetamine sulfate were stable for 8h, and HMMA sulfate and MDA (102 ± 51%) for up to 24h. The high variance observed for MDA could be due to N-dealkylation from MDMA or MDEA in some samples. For MDEA and 4-hydroxy-3-methoxyethylamphetamine, data could not be assessed due to concentrations lower than LOQ in the incubations, but occasionally higher than LOD. Pooled human urine and urinated soil was applied to study the stability of human urinary metabolites. HMMA could serve as a new target for quantitative assessment of MDMA consumption by wastewater analysis and allow distinguishing between consumed and discharged MDMA.

MOP 4 **METABOLISM OF THE DESIGNER DRUG PMMA (4-METHOXYMETHAMPHETAMINE) IN HUMAN LIVER MICROSOMES**

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The main metabolism of PMMA most probably occurs by the polymorphic CYP2D6 enzyme, forming the active metabolite 4-hydroxymethamphetamine (OH-MA, pholedrine), in addition to minor metabolic pathways forming PMA (paramethoxyamphetamine). Current knowledge on the human PMMA metabolism is very limited. The hitherto known PMMA metabolites are also formed from MDMA and MA, except for PMA (4- methoxyamphetamine), which is unique to PMMA.

The aim of the present work was to study the in vitro metabolism of PMMA in human liver microsomes, investigating which metabolites are formed and if there is a major impact of the CYP2D6 genotype, compared with the reference drugs methamphetamine (MA) and MDMA (methylenedioxymethamphetamine).

The metabolite formation was measured after 0, 15, 30, 60, 120, 240 and 360 minute incubation at 36.5 °C of 100 µM of PMMA, MA or MDMA with genotyped human liver microsomes classified as CYP2D6 ultrarapid (UM), pooled or poor (PM) metabolizers, using n=4 experiments (microsomes from 2-3 individuals, or from 3 LOTS of 200 pooled individuals) per time point, applying LC-MS/MS analysis.

PMMA was metabolized (in decreasing order) to OH-MA, PMA, 4-hydroxyamphetamine, and dihydroxymethamphetamine (di-OH-MA); MA was metabolized to amphetamine, OH-MA, and di-OH-MA; and MDMA was metabolized to di-OH-MA and MDA. The formation of di-OH-MA from PMMA, A, and MDMA was 0-2.2%, 0-0.1% and 2.2-5.4%, respectively, and the maximum concentrations of di-OH-MA were reached within 120-360 minutes. The CYP2D6 genotype had a major impact on the metabolism of PMMA; after 360 minutes of incubation, 84 – 53 – 25% of PMMA was metabolized in the UM-, the pooled-, and the PM genotypes, respectively, and mainly to 4-OH-MA. The impact of CYP2D6 genotype on the metabolism of MA was minor, 10 – 7 and 4%, respectively, while the impact on the metabolism of MDMA was moderate, 35 – 25 – 18%.

The major metabolite formed from PMMA in human liver microsomes was 4-OH-MA. The degree of formation was strongly correlated with the CYP2D6 genotype, from high-grade conversion in the CYP2D6 UM microsomes to low-grade in the PM microsomes. The neurotoxic metabolite di-OH-MA was formed from PMMA and from MA, as well as from MDMA, but the concentrations measured were low. Di-OH-MA has not formerly been reported as a metabolite of PMMA or MA, but it is a well-known reactive catechol metabolite of MDMA.

MONDAY, August 31st

MOP 5 METABOLISM OF SYNTHETIC CANNABINOIDS - SIMILARITIES AND DIFFERENCES BETWEEN METABOLIC PATTERNS OF DIFFERENT STRUCTURAL SUBCLASSES

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The market of new psychoactive substances is still highly dynamic. In 2014 a flood of new synthetic cannabinoids were reported to drug monitoring agencies. Next to substances with well-known patterns of structural modifications, also compounds of two completely new structural classes carrying either a valine-derived or a cumyl substituent, were identified.
For each new substance identified in 'legal high' products or detected in authentic serum samples we aimed to develop a robust method for the detection of the parent compounds and their main metabolites in urine samples.
For identification of the main metabolites an in vitro assay using pooled human liver microsomes was applied. Analytical methods used for these studies comprised LC-ESI-MS/MS and LC-ESI-Q-ToF-MS. If available, authentic urine samples were analyzed to compare in vitro and in vivo metabolic patterns.
The in vitro main metabolites of 5F-ABICA, 5F-AB-PINACA, 5F-ADBICA, 5F-AMB, 5F-Cumyl-PINACA, 5F-SDB-005, 5F-SDB-006, AB-CHMINACA, AB-FUBINACA, AB-PINACA, AMB, BB-22, FUB-AMB, FUB-PB-22, Cumyl-PINACA, MDMB-CHMICA, MMB-2201, NM-2201, NNEI, SDB-005 and SDB-006 were characterized. Comparing the metabolic patterns of structurally related compounds, some general rules of metabolic transformation are suggested: Indole and indazole carboxyesters as well as the primary amide or methoxyester group of the valine-derived moieties are likely to undergo hydrolysis. Alkyl side chains such as pentyl or cyclohexyl methyl are highly prone to hydroxylation, often leading to sets of isomers. Fluorinated pentyl side chains mostly show hydrolytic defluorination. For AB-CHMINACA, AB-FUBINACA, AB-PINACA, Cumyl-PINACA, FUB-AMB and MDMB-CHMICA the in vivo main metabolites were also identified and were in good agreement with the in vitro results.
Increasing knowledge on the metabolism of individual compounds enables more reliable prediction of metabolic profiles of new, structurally related compounds. Metabolic reactions can lead to identical main metabolites for several compounds, and these can be very valuable targets for general unknown screening procedures. Nevertheless, in a forensic context it is often necessary to identify the consumed compound by detecting specific metabolites. Mass spectrometric data of metabolites obtained from in vitro assays are of great value for method development, particularly when reference material is not yet commercially available.

MOP 6 METABOLIC STUDIES OF SYNTHETIC CANNABINOID PB-22 BY CUNNINGHAMELLA ELEGANS

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As new synthetic cannabinoids are constantly emerging in the market, it is important to investigate the metabolic profile for drug testing purposes.
This study intends to identify the metabolite profile of PB-22 produced by the fungus, Cunninghamella elegans, which has previously demonstrated the ability to produce metabolites of synthetic cannabinoids, JWH-018, JWH-073 and AM2201, in a similar manner to in vivo human and in vitro human liver microsomes (HLM) studies.
C. elegans cultures were grown on potato dextrose agar plates at 27 °C for 5 days. The mycelia of the fungus were transferred into 250 mL conical flasks containing 100 mL each of liquid media composed of glucose, glycerol, peptone, yeast extract, KH_2PO_4 and NaCl in distilled water. The flasks were incubated for 48 h at 26 °C on a rotary shaker at 180 rpm. Then, PB-22 was added to the flasks and incubated for another 72 h. The solution was filtered and extracted with dichloromethane. The extract was then dried and reconstituted into acetonitrile. The resulting metabolites were analysed by LC-MS/MS and HR-MS/MS techniques. Full scan and product ion scan were obtained in positive ion mode. Control samples consisting of fungus in media and drug in media were also analysed.
LC-MS/MS and HR-MS/MS analysis detected the formation of several metabolites including ester hydrolysis products (pentylindole-3-carboxylic acid) with and without monohydroxylation, monohydroxylation, dihydroxylation and dihydrodiol metabolites. These results are comparable with human hepatocyte and HLM studies.
The fungus C. elegans has been demonstrated to produce metabolite signature which correlates well with human hepatocyte and HLM studies. C. elegans model thus appears to be a promising platform for investigating synthetic cannabinoid metabolism. Furthermore, the ease of scaling up with this fungus model has the potential to allow structural characterisation of major metabolites by NMR and/or production of reference materials.

MOP 7

STUDY ON BEHAVIORAL AND PHARMACOKINETIC PROPERTIES OF 5,6-Methylenedioxy-2-aminoindane (MDAI) INCLUDING BIOTRANSFORMATION IN RATS AFTER A SUBCUTANEOUS BOLUS DOSE

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The aminoindanes are likely to produce the empathogenic and entactogenic effects of serotonin releasing drugs and unexpected toxic consequences can appear. MDAI was the subject of our experimental study because little is known on psychopharmacological or toxicological properties and metabolism of this drug so far.
a) To develop the LC/HRMS method to study the disposition of MDAI in the blood and the brain of experimental rats b) To evaluate the pharmacokinetic profile in comparison with behavioral data c) To identify the main metabolites of MDAI in urine using combination of LC/HRMS and GC/MS methods
All experiments were performed with male Wistar rats weighing 200-250 g. a) In behavioral experiments MDAI 5, 10 and 20 mg/kg sc. was administered; the locomotor activity in the open field (Ethovision, Noldus) and effect on sensorimotor gating in the test of prepulse inhibition of acoustic startle reaction (PPI, SR-Lab) was evaluated. Furthermore a rectal temperature of animals housed in groups and separately was also evaluated. b) In pharmacokinetic experiments MDAI 10 mg/kg sc. was administered, animals were decapitated (n=8) at specified times and blood and whole brain were collected. The analyses were performed using Dionex Ultimate 3000 UHPLC coupled to an Exactive Plus-Orbitrap MS. c) For the biotransformation study, 3 rats were administered MDAI 20 mg/kg sc. and excreted urine was collected for 24 hours. In searching for MDAI metabolites LC-HRMS screening method was used in combination with GC/MS (Agilent HP6890/5973).
Behavior: MDAI increased locomotor activity in all doses used and also disrupted PPI. The effects were most robust when the drug was administered 15 min prior testing and tended to worn off 60 min after the administration especially with the lowest dose used. The drug also significantly increased rectal temperature of about 2°C and induced profound perspiration in animals housed in groups but not in those housed separately. Pharmacokinetics: Maximum median MDAI serum concentration 4.3 mg/L (SD 0.8 mg/L) was attained in 30 min after the s.c. dose with estimated elimination half-life 0.8 h. The influx into the brain tissue was not delayed, the maximum median concentration in the brain 18.2 mg/L (SD 1.4 mg/L) was reached in 30 min after the dose. The brain to serum ratio was close to 4 through the whole temporal observation. Excretion into urine: MDAI was excreted into the urine in its parent form accompanied by the main metabolite 5-hydroxy, 6-methoxy-aminoindane. This metabolite was confirmed using the synthesized reference standard.
MDAI subcutaneous dose 10 mg/kg indicated high and rapid bioavailability, with high rate of elimination, rapid and massive influx into the brain tissue with outlasting brain to serum ratio close to 4. The behavioral parameters corresponded to a profile of a serotonergic drug comparable to MDMA. The increased perspiration in animals housed in groups may indicate its increased relative risk to induce serotonin syndrome. The peak of behavioral effects corresponded to maximum serum and brain levels. This work was supported by projects MICR VG20122015075 and ED2.1.00/03.0078.

MONDAY, August 31st

11.30 am – 1.00 pm SESSION 2 – Alcohol Use Markers

MOP 8 ETHYL 4-HYDROXYBUTYRATE – SEARCH FOR A LONG-TERM MARKER OF COMBINED GAMMA-BUTYROLACTONE (GBL) AND ALCOHOL UPTAKE

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A common way of drug application in drug facilitated crimes (DFC) is spiking an alcoholic beverage with gamma-butyrolactone (GBL). After spiking, gamma-hydroxybutyrate (GHB) will be formed by hydrolysis and under certain conditions ethyl 4-hydroxybutyrate, the ethyl ester of GHB (GHB-Et), can be generated. Furthermore, GHB Et might be formed in-vivo. Similar observations have previously been made for the combined intake of ethanol and cocaine or methylphenidate. Although esters are often rapidly cleaved by carboxylases, inhibition of human carboxylesterase-1 by ethanol might slow down this process significantly.
Aim of the present study was to investigate if GHB Et can be detected in serum after the intake of GBL and ethanol and to assess if this analyte might extend the detection window of a GBL administration.
One participant drank 150 ml vodka mixed with bitter lemon and afterwards a drink containing 50 ml vodka, 2.9 ml GBL and bitter lemon. In a second experiment, three volunteers ingested 1 ml GBL and/or 100 ml rum with 100 ml cola. Serum samples were collected in both experiments on a regular basis. Additionally, serum samples with alcohol concentrations of approximately 1.2 g/l were spiked with each 100 µg/ml GBL and GHB. The samples were stored for 5 days at room temperature to assess in-vitro formation of GHB Et. All serum samples were analyzed for GHB Et and GHB after protein precipitation applying an LC-MS3 and LC-MS/MS method, respectively (LOD: GHB Et: 1.0 ng/ml; GHB: below 1.0 µg/ml).
GHB Et was detected in serum samples obtained between 18 min and 3.4 h after high dose intake of ethanol and GBL. However, the window of detection for GHB was identical in this experiment. The highest GHB concentration detected was 177 µg/ml in the serum sample obtained 53 min after the intake and the highest GHB Et concentration of approximately 23 ng/ml was detected in the serum sample collected 73 min post intake. No GHB Et was detectable after sole intake of GBL or ethanol. GHB Et concentrations of 1.5 2.3 ng/ml were detected in samples obtained 40 – 105 min post intake of GBL and ethanol (low dose). Furthermore, no detectable amount of GHB Et was formed in the stored serum samples indicating that either uptake of ester in the beverage and/or in vivo formation could explain the presence of GHB Et.
Detection of GHB Et in serum might be a potential marker for ingestion of alcoholic beverages spiked with GBL. First studies indicate that the window of detection of a GBL administration cannot be extended by this analyte. However, further studies are needed to evaluate the pharmacokinetic parameters of this compound in particular in the presence of ethanol. Additionally, it needs to be assessed if uptake of GHB Et is mandatory for detection of this compound or if formation occurs also in vivo.

MOP 9 FORMATION AND INHIBITION OF ETHYL GLUCURONIDE AND ETHYL SULFATE

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Ethyl glucuronide (EtG) und ethyl sulfate (EtS) are widely accepted alcohol biomarkers in forensic and clinical settings. However, controlled studies suggest an inter-individual variation of urinary EtG and EtS, which warrants further explanation.
Therefore, kinetic and inhibition studies using recombinant glucuronosyltransferases (UGTs) and sulfotransferases (SULTs) were performed to characterize enzymes involved in the formation of EtG and EtS and to test polyphenols for their inhibitory potential. Quercetin, kaempferol and resveratrol being abundant in food but also in alcoholic beverages were chosen for inhibition experiments. Human liver microsomes (HLMs) und human liver cytosol preparations (HLCs) were also characterized with respect to EtG and EtS formation allowing an in vitro-in vivo approach.
After optimization of incubation procedures, major UGTs and SULTs catalyzing formation of EtG and EtS were identified. Subsequently, respective kinetics were established, that followed inhibition experiments using quercetin, kaempferol and resveratrol. In addition, incubations with HLMs and HLCs were performed. Analysis was by liquid chromatography/tandem mass spectrometry following solid phase extraction for EtG due to severe matrix effects and by direct injection for EtS.
The maximum EtG and EtS formation rates were observed with HLMs and SULT1A1, respectively. All kinetics could best be described by Michaelis-Menten kinetics. Resveratrol was a competitive inhibitor of UGT1A1, UGT1A9 and HLMs; quercetin and kaempferol were inhibitors of all transferases under investigation except UGT2B15. Findings for quercetin with regard to UGT2B7 and SULT2A1 and for kaempferol with regard to SULT1E1 and SULT2A1 suggest a mechanism based inhibition.
Major isoforms being responsible for EtG and EtS were identified. Prediction of the inhibitory potential indicates that nutritional components such as polyphenols may contribute to the variable formation rate of both, EtG and EtS. A possible contribution due to the known polymorphisms of UGTs and SULTs needs further appraisal.

MOP 10 **DEVELOPMENT OF A STANDARDISED TEST FOR THE ACTIVITY OF THE ENZYME PHOSPHOLIPASE D (PLD), RESPONSIBLE FOR BIOSYNTHESIS OF THE ALCOHOL BIOMARKER PHOSPHATIDYLETHANOL (PETH)**

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Phosphatidylethanol (PEth) is a direct alcohol biomarker, which is highly specific for the detection of alcohol consumption as PEth is only formed from phosphatidylcholine (PC) by the enzyme phospholipase D (PLD) in the presence of ethanol. So far PEth was only used to differentiate between social drinking and alcohol abuse, but a number of drinking studies showed that also a single alcohol intake up to a blood alcohol concentration (BAC) of 1 g/kg can be detected via PEth determination.
Such a drinking study with 16 volunteers revealed individual differences in maximum PEth levels after drinking up to a BAC of 1 g/kg. This seemed to be due to different PLD activities in the tested persons. Post-sampling formation of PEth was shown to occur in blood samples, which still contained alcohol. Therefore, a standardised test for individual PLD activities was developed.
For this test, fresh PEth-negative blood samples were collected from a volunteer. Ethanol was added in different concentrations (0.1 – 3 g/kg BAC) directly after blood sampling. The specimens were incubated at 37 °C. Aliquots were sampled every hour on the first day and once daily on subsequent days. PEth concentrations were determined by online-SPE-LC-MS/MS.
PEth formation was linear in the first 7 hours of incubation and dependent on alcohol concentration. The higher the BAC, the more PEth was formed. The velocities of PEth 16:0/18:1-formation were 1.5 $ng \cdot mL^{-1} \cdot h^{-1}$ (BAC 0.1 g/kg), 11.4 $ng \cdot mL^{-1} \cdot h^{-1}$ (1 g/kg), 17.4 $ng \cdot mL^{-1} \cdot h^{-1}$ (2 g/kg) and 20.2 $ng \cdot mL^{-1} \cdot h^{-1}$ (3 g/kg). For PEth 16:0/18:2-formation, the velocities were 1.7 $ng \cdot mL^{-1} \cdot h^{-1}$ (BAC 0.1 g/kg), 13.3 $ng \cdot mL^{-1} \cdot h^{-1}$ (1 g/kg), 17.1 $ng \cdot mL^{-1} \cdot h^{-1}$ (2 g/kg) and 21.1 $ng \cdot mL^{-1} \cdot h^{-1}$ (3 g/kg). Under these conditions, PLD was active for 3 days. Maximum concentrations reached 431 ng/mL (PEth 16:0/18:1) and 496 ng/mL (PEth 16:0/18:2) at 3 g/kg.
PLD activity is dependent on the BAC. As a consequence, it is essential to inhibit PLD activity after blood collection to avoid post-sampling formation of PEth in blood samples with a positive BAC.

MOP 11 **OBSERVATIONS ON THE SAMPLING OF NON-VOLATILES IN EXHALED BREATH – DETECTION OF PHOSPHATIDYLETHANOL**

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Exhaled breath contains aerosol particles consisting of fluid (surfactant) material from terminal parts of the respiratory system and is proposed as a non-invasive specimen for drug testing. The airway lining fluid is rich in phosphatidylcholines (PC), however with a different species composition compared with other parts of the body. Phosphatidylethanol (PEth) formed from PC in the presence of ethanol, which is used as an alcohol biomarker, might therefore be measurable in breath following alcohol intoxication. Additional experiments with methadone and codeine was performed to better understand the sampling procedure.
To further study the sampling of non-volatiles from exhaled breath.
Validated LC-MS/MS-SRM-methods for quantitative determination of PC, PEth, methadone and codeine in breath samples were used. Sampling of exhaled breath was done using the SensAbues disposable device, which collects particles from about 30 L of exhaled breath. Breath samples were collected from healthy volunteers, patients on methadone treatment, and from patients recovering from acute alcohol intoxication. In one experiment, healthy volunteers had a codeine solution in the mouth for 1 min prior to exhaled breath sampling. Oral fluid was also collected using the Quantisal device.
PC analysis in exhaled breath demonstrated that PC 16:0/16:0 dominated over PC 16:0/18:1 (ratio 1.9, n=11), whereas the reversed was found in oral fluid (ratio 0.15). PEth 16:0/18:1 was detected in exhaled breath from heavy alcohol drinkers (20-77 pg/filter, n=12) but not in social drinkers used as controls (<2 pg/filter). In samples collected from methadone patients, analysis of methadone in different parts of the SensAbues device demonstrated that only a fraction of the exhaled methadone was found on the filter (about 20% of that passing through the mouth piece). When having a strong solution of codeine in the mouth prior to sampling, codeine was measurable in the breath samples collected from 2 out of 5 individuals.
The dominance of PC 16:0/16:0 in the exhaled breath samples is consistent with the PC composition of surfactant and confirms that the collected aerosol particles predominantly originate from the lung and not due to contamination by oral fluid. However, based on the experiments with codeine, some contamination from the oral cavity can occur. The investigation of methadone in different parts of the sampling device showed that particles deposit also on the inner surface of the manifold, suggesting there is room for improvement of the sampling procedure. PEth was detected in exhaled breath from heavy drinkers, but the dominance of PEth 16:0/18:1 indicated that it did not originate from PC in the surfactant phase.

MONDAY, August 31st

MOP 12 AUTO-BREWERY SYNDROME AS A POSSIBLE EXPLANATION FOR INCREASED ALCOHOL BIOMARKERS?

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A man who had lost his driving license because of driving under the influence of alcohol asked for help. He argued that even though he abstained from alcohol, his carbohydrate-deficient transferrin levels (%CDT) over the last two years (n=12) were all positive and, for this reason, his driving license was not reissued by the authorities. Two other blood biomarkers for chronic drinking, GGT and MCV, were always normal. The man is a successful ultra-distance runner which seemed difficult to bring into agreement with daily drinking. He had undergone tests at gastroenterology units without clinical findings. We monitored this individual for one year (May 2013 to June 2014) with different alcohol biomarkers.
The study aimed to find possible alternative explanations except alcohol for his elevated %CDT values.
All samples (47 urine, 54 serum, 42 EDTA whole blood, 1 hair, and 3 stool samples) were collected at the Salzburg laboratory. Stool cultures were performed in a microbiology laboratory. Urinary sucrose was analysed by UPLC-MS/MS (cutoff 0.5 µg/mL), ethanol in serum and urine by GC-FID, %CDT in serum by an HPLC-UV commercial kit (Chromsystems; cutoff 2%), ethyl glucuronide (EtG) in urine (cutoff 100 ng/mL), serum (cutoff 1 ng/mL), and 3 cm of proximal head hair by UPLC-MS/MS (cutoff 7 pg/mg), and phosphatidylethanol (PEth 16:0/18:1; cutoff 0.2 µmol/L) in whole blood by LC-MS/MS. All urine and serum samples collected during the 1-year observation period were negative for ethanol, whereas all blood samples tested positive for %CDT and PEth. The CDT and PEth curves showed a similar time-course and also paralleled the EtG concentrations. In May 2013, 3 stool samples collected on consecutive days all contained high amounts of the ethanol-producing yeast Candida albicans. When asked about his diet, the man claimed he regularly consumed large amounts of chocolate and sucrose- and glucose-rich "Ensure Plus" drinks (20.2 g/100 mL; Abbott). Taken together, this suggested he might suffer from "auto-brewery" syndrome. When asked to refrain from the carbohydrate-rich diet, sucrose was only detectable in urine in early-June 2013 and on 2 consecutive days in September 2013. In July 2013, he was treated with Nystatin and Mutaflor to re-colonize the gut. Stool cultures collected after this treatment were negative for C. albicans and 3 urine samples tested negative for EtG. In September-October 2013, several urine and serum samples (n=9) were also EtG negative and at the same time his lowest %CDT and PEth values were obtained. However, from December 2013 and onwards the values increased, probably because he had begun to eat a carbohydrate-rich diet. A hair sample collected in May 2014 contained 190 pg EtG/mg hair. A stool culture was negative for yeasts but instead contained Enterobacteriaceae which can also produce ethanol.
The possibility of endogenous ethanol fermentation, also known as auto-brewery syndrome, in combination with an extreme carbohydrate diet could possibly explain the increased alcohol biomarkers. The reason no ethanol was detectable in serum and urine might be first pass metabolism in the liver.

MOP 13 A NOVEL, SIMULTANEOUS EXTRACTION OF FAEE AND ETG FROM MECONIUM AND ANALYSIS BY LC-MS/MS

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Fatty acid ethyl esters (FAEE) and ethylglucuronide (EtG) in meconium have been widely studied as biomarkers of maternal alcohol consumption during pregnancy. Many analytical approaches have been proposed for their analysis, mainly consisting in separated extraction procedures, requiring in general the use of two meconium aliquots.
This study aims to validate a new analytical procedure for simultaneous extraction of FAEE and EtG from the same meconium aliquot through a single solid-phase extraction (SPE), followed by liquid chromatograph-tandem mass spectrometer (LC-MS/MS) detection.
Targeted FAEE were: ethylmyristate (MIR), ethylpalmitate (PAL), ethyloleate (OLE) and ethylstearate (STE). 200 mg of meconium were added with acetonitrile (ACN) and then sonicated for 15 min. SPE was performed by means of aminopropyl columns (Bond Elut NH_2) previously conditioned with methanol, water and ACN. FAEE elution was achieved with hexane, followed by EtG elution with H_2O. The two solutions were dried under nitrogen stream and then reconstituted with 50 µL of ACN and 50 µL of methanol, respectively. FAEE were detected in LC-MS/MS on a triple quadrupole in positive ESI mode with a Zorbax C8 analytical column. Elution was performed starting from a 30:70 aqueous 5mM acid formic (A) : ACN (B) mobile phase, increasing to 90% B over 3 min, held for 4 min. Deuterated analogues of FAEE (MIR-d5, m/z 262; PAL-d5, m/z 290; OLE-d5, m/z 316; STE-d5, m/z 318) were used as internal standard (IS). FAEE transitions for multiple reaction monitoring (MRM) acquisition were: m/z 257→57,88, MIR; m/z 285→57, 72, PAL; m/z 311→72, 114, OLE; m/z 257→57, 72 STE. EtG analysis was performed in negative ionization mode using a Zorbax C18 column run isocratically at 1% B (run time: 1 min; rinsing step at 90% B for 2 min). The following MRM transitions were adopted: m/z 221→75, 85 EtG; m/z 226→75, 85 EtG-d5 (IS).
Since no FAEE free meconium is in principle available, the standard addition method was used to collect blank samples for the validation. Regarding FAEE, linearity was evaluated in the range 10 - 5000 ng/g for each ester (R^2 from 0.9961 - 0.9984). Limit of quantification (LOQ) and limit of detection (LOD) were preliminary estimated for all esters in 10 and 5 ng/g, respectively. For EtG, linearity along the calibration range 10 - 500 ng/g was fulfilled (R^2 0.9987). LOQ and LOD were estimated in 10 and 7 ng/g respectively.
This novel procedure is a breakthrough in meconium detection of FAEE and EtG as it allows their extraction from the same meconium sample through a single SPE, providing a direct comparison of these markers. Other advantages are the reduction in time consuming and costs for the sample preparation phase. This method will be applied to a pilot study on fetal alcohol spectrum disorders.

MOP 14 **KINETICS OF ETG IN FINGERNAILS IN A GROUP OF CHRONIC HEAVY DRINKERS**

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EtG in fingernails has been recently studied as potential biomarker for the detection of moderate as well as chronic alcohol consumption. The mechanism of incorporation of EtG in keratin matrices is already known, though remains unclear whether it could be removed by routine hygiene practice.
The aim of this study was: a) to detect and quantify EtG in fingernails (NEtG) and hair (HEtG) in a group of chronic alcohol heavy drinkers at the beginning of a withdrawal treatment: b) to evaluate NEtG stability, by measuring nails during alcohol abstinence every 7-10 days for up to two months, by clipping a new overhang excess each time.
Excess overhang of nails and the 3-cm proximal hair segments were extracted in 700 µL bidistilled water. D_5-EtG was used as internal standard. A C18 column was used for isocratic separation of EtG from the keratin matrix. The method were developed in multiple reaction monitoring (MRM) in negative mode. A questionnaire, together with the informed consent, was submitted to all the participants, in order to evaluate alcohol intake within the last months before sample collection. 15 heavy drinkers joined this study. At the beginning of the withdrawal treatment HEtG mean level was 132.8 pg/mg (median: 50.5 pg/mg), while NEtG provided higher concentrations with a mean value of 435.5 pg/mg (median: 257.4). Hair results for 5 out of 15 cases were under the internationally accepted cutoff of 30 pg/mg; this could be caused by the fact that alcohol consumption was terminated up to two weeks before sample collection. The mean NEtG/HEtG ratio was 4.9 ± 4.0 (median: 2.8), thus assessing that EtG is accumulated in fingernails in greater amount than in hair. Nails were measured every 7-10 days for up to ten weeks. It was observed that NEtG decreasing started from the first week of abstinence, disappearing from the fingernails within a period ranging from two to six weeks depending on the initial level.
This study showed a higher sensitivity of NEtG respect to HEtG in evaluating either chronic excessive as well as moderate alcohol consumption habits. The relatively rapid decreasing of EtG from keratin matrix is of concern and could be due to the hydrophilicity of this compound. This trend should be now evaluated in other keratin matrices, like toenails.

2.30-4.00pm SESSION 3 – New Psychoactive Substances (intoxication cases and identification)

MOP 15 **DETERMINATION OF NEWLY ENCOUNTERED DESIGNER DRUGS α-PHP AND ACETYLFENTANYL IN AN ACUTE INTOXICATION CASE BY LC/Q-TOFMS**

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Although the designer drugs have been strictly banned in Japan, acute poisoning cases by overdose of new designer drugs have occurred. It is more difficult to detect designer drug in biological specimens because some new designer drugs can effect on human body at low dose or may show short half-life in blood. Forensic toxicologists must exceed such difficulties and accumulate the information about acute poisoning cases of newly encountered designer drugs. We applied screening test by LC/Q-TOFMS to postmortem blood sample, resulting in detection of a newly encountered synthetic cathinone, α-PHP, and acetylfentanyl. We measured their concentration in blood. Also, we investigated their main metabolic pathways in human.
Samples: Urine and blood were obtained at an autopsy. Screening test by LC/Q-TOFMS was as follows; Sample preparation: Methanol was added to blood for deproteinization. After centrifugation, upper layer was removed and evaporated to dryness. The residues were resolved in 20% methanol - 10mM ammonium acetate buffer. LC/QTOF-MS systems: LC/QTOF-MS was performed on a NexeraX2 (Shimadzu) and TripleTOF5600 system (AB SCIEX). Separation was performed with a L-column 2 ODS (particle size 3 µm; 150×1.5 mm i.d.; Chemicals Evaluation and Research Institute, Tokyo, Japan). The mobile phases A and B were as follows; A: 5% methanol-10 mM ammonium acetate (AAc) buffer and B: 95% methanol-10 mM AAc buffer. Flow rate was set at 0.10 mL/min. Electrospray ionization was performed in the positive mode. Quantitative analysis was as follows; Quantitation of α-PHP and acetylfentanyl in blood was executed by standard addition method. Sample preparation: Standard solutions of α-PHP and acetylfentanyl was added to postmortem blood and extracted with ethyl acetate (EA) under alkaline condition. After centrifugation, extracts were evaporated under gentle nitrogen stream to dryness. The residues were reconstituted by EA. GC/MS systems: GC/MS-QP2010 Plus (Shimadzu) was used. A GC capillary column Rix-5Sil MS (0.25 mm i.d. × 30 mm; 0.25 µm; Restek) was used for separation. Electron ionization was used and Selected ion monitoring (SIM) mode was used for quantitation.
LC/Q-TOFMS screening with information dependent acquisition mode could detect α-PHP and acetylfentanyl in blood. Quantitative analysis revealed that blood concentration level of α-PHP and acetylfentanyl were 413 and 69 ng/mL, respectively. Accurate mass measurement for searching main metabolites could detect some metabolites, and expected metabolic pathways are as follows; hydroxylation, reduction of β-keto moiety, and 2"-Oxidation for α-PHP, and hydroxylation and deacetylation for acetylfentanyl.
This study suggests that combination use of α-PHP and acetylfentanyl can contribute to victim's death. In this presentation, we will demonstrate analytical results of urinary metabolites with or without enzymatic hydrolysis.

MONDAY, August 31st

MOP 16 THREE DEATHS INVOLVING SYNTHETIC CANNABINOID- PB-22 (1-PENTYL-8-QUINOLINYL ESTER-1H-INDOLE-3-CARBOXYLIC ACID)

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Over the last 5 years there has been an influx of novel designer drugs (mostly illegal), which are intended to mimic the effects of cannabis (synthetic cannabinoids). PB-22 (1-pentyl-8-quinolinyl ester-1H-indole-3-carboxylic acid) is a relatively new synthetic cannabinoid, which has cannabis like activity and possibly other as yet unknown effects. There is no published data on the pharmacodynamics or pharmacokinetics of PB-22. Although synthetic cannabinoids have been reported in association with sudden death, the precise pathophysiological mechanisms by which death occurs remain unclear.
To present 3 deaths reported to the coroner involving PB-22.
The cases were subject to full autopsy and toxicological examination. The cases were subsequently screened for a number of synthetic cannabinoids based on a previously published method.
There were 3 cases identified over a four month period where the cause of death was unascertained following a full autopsy and routine forensic toxicology. PB-22 was identified as the main toxicological finding in all three cases. The concentration ranges detected in femoral blood were 0.2, 0.5 and 3 ng/mL. All 3 deaths occurred at home in the setting of using this drug with no other cause of death identified. Drug paraphernalia were present in 2 cases. One case had plant matter labelled "Passion Flower Herb – Zonk" which was analysed and found to contain PB-22. Another case had a number of exhibits at scene including: various plant matter, some with labels such as "Stoner Pot-Pourri K11" and "Supanova Pot-Pourri" as well as a smoking pipe. These exhibits were analysed and were found to contain a variety of synthetic cannabinoids including: PB-22, 5F-PB-22, XLR-11, BB-22, JWH-122, UR-144, AM-2201 and MAM-2201. 5F-PB-22, the 5-fluoro analog of PB-22 has been reported previously in unexpected deaths. The subsequent identification of PB-22 raises the question of its possible involvement in the deaths, particularly in the absence of other significant drugs or competing cause of death.
The risk of using synthetic cannabinoids has not been clearly established and while we remain unsure about the exact role of PB-22 in these 3 deaths, persons with an existing heart condition may be at risk of sudden death when using drugs that can produce unwanted increases in heart rate and blood pressure. Synthetic cannabinoids have also been associated with seizures and one of the cases reported here had a history of epilepsy.

MOP 17 DEATH AFTER CONSUMPTION OF NEW SYNTHETIC CANNABINOIDS AND ALCOHOL

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A 33 year old man was found dead sitting in a friends' flat in front of a sofa in his vomit. He had come to his friend after staying in some taverns where he drank alcohol. The friend left the flat to go on errands and as he came back a few hours later he found his companion dead. The deceased was known to drink a lot of alcohol and consume so called legal highs containing synthetic cannabinoids. Ascertain the cause of death.
The case was subject to a full medicolegal autopsy and subsequent toxicological examination. This involved an immunoassay screen in urine and blood for opiates, methadone, buprenorphine, benzodiazepines, amphetamines, methamphetamines, cannabinoids, and cocaine metabolites. Extracts of urine, blood, and gastric content were screened by GC-MS in scan mode. Blood was additionally analysed by LC-MS/MS for more than 100 active pharmaceuticals in a multi target method and by another method for synthetic cannabinoids. Gastric content was proofed for volatile compounds and insecticides. Drugs of significance were quantified in heart blood, femoral blood, brain, fatty tissue, and pubic hair using LC-MS/MS.
Pathological findings included oedema of the inner organs and respiratory tract infection. Neither aspiration of gastric content, nor injection sites, signs of defence, or injuries could be observed. Toxicological results of the peripheral blood sample were 0.265% alcohol and 0.85 ng/ml 5F-PB-22. Further substances were found as follows. Urine: mirtazapine, traces of 5F-PB-22, 0.375% alcohol; Heartblood: traces of mirtazapin, 0.29 ng/ml 5F-PB-22; Brain: 8.0 ng/g 5F-PB-22, 0.16 ng/g JWH-210; Pubic hair: 0.16 ng/mg EAM-2201, 1.0 ng/mg THC, hints for JWH-210; Fatty tissue: 0.58 ng/g RCS-04, 0.04 ng/g JWH-122, 1.15 ng/g JWH-210, 1.29 ng/g EAM-2201, 2.71 ng/g 5F-PB-22, 241 ng/g THC.
Blood concentrations of 5F-PB-22 in lethal cases were described in literature ranging from 1.1 to 1.5 ng/ml. In our case the concentration of 5F-PB-22 in peripheral blood was slightly beyond the literature values. The detection of further substances in hair and especially fatty tissue revealed that the decedent had consumed other synthetic cannabinoids prior to death which may have resulted in adaptation to this class of drugs. Nevertheless, excluding a death due to an internal condition or external violence, the cause of death was combined intoxication.

MOP 18 **A FATAL INTOXICATION RELATED TO MDPV AND PENTEDRONE COMBINED WITH ANTI PSYCHOTIC AND ANTIDEPRESSANT SUBSTANCES IN CYPRUS**

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Cathinone derivatives frequently appear in the recreational drug market as "legal highs" and they have quickly emerged worldwide as the latest in drug of abuse. MDPV (3,4-methylenedioxypyrovalerone) is a cathinone derivative, so called designer drug and is among the ingredients listed in bath salts with stimulant effects similar to cocaine and amphetamines. Pentedrone is also a designer drug with presumably stimulant effects which has been found since 2010 in bath salts mixes. Both of the substances are prohibited in Cyprus according to the Generic Legislation introduced in 2011 to ban NPSs.
This study presents the investigation of the first death in Cyprus, involving the drugs MDPV, Pentedrone, Olanzapine, Mirtazapine, Etizolam and Ephedrine. A 42 year old Cypriot man with a history of serious psychiatric problems (attempted suicide twice in the past), was found dead in his bed. Seized materials (traces of unknown powder and tablets) were also found closed to his body.
Routine toxicological procedure was followed in blood and urine. Stomach content was not submitted. MDPV was obtained from The National Measurement Institute of Australia. Mirtazapine was purchased from Lipomed. Olanzapine and Ephedrine were purchased from Cerilliant. Pentedrone and Etizolam were not available in lab by that time, thus identification was based on mass spectrum of SWG DRUG Library. Immunological screening with VIVA-E Analyzer (Siemens) was carried in urine to test for DOA and Benzodiazepines. The test was positive for Amphetamines (mainly due to the presence of ephedrine) and for Benzodiazepines. It is well-known that standard screening procedures fail to detect MDPV and most other designer drugs. Sample cleanup was performed by SPE cartridges and the confirmation on the SHIMADZU GC-MS, QP 2010 (EI mode, column HP-5 MS- UI, 30 m x 0,25 m x 0,25 μm). MDPV was detected following an alkaline extraction without the need for derivatisation. A second alkaline SPE extraction was carried out and analyzed with different chromatographic conditions to test for any other medicinal drugs present. Pentedrone, along with Etizolam, Olanzapine and Mirtazapine were detected. No blood alcohol was detected.
The confirmation results and quantitative analysis showed the presence of MDPV in blood and urine (0,046mg/L and 1,3mg/L respectively), Olanzapine (4,2mg/L , fatal), Mirtazapine (0,57mg/L), and Ephedrine (0,068mg/L), Pentedrone and Etizolam.
At the time of submission this was the first reported death related with MDPV and Pentedrone and the case reported to the EMCDDA Early Warning System. The two cathinones as already mentioned were not the sole substances detected and the toxic levels of MDPV are not reported in the literature so far. However, the high levels of olanzapine and the co-exist of Mirtazapine and Etizolam among with the two designer drugs, might had contributed to the death of the 42 year old man. The death was classified as an accident due to polydrug intoxication.

MOP 19 **MEPHEDRONE IN POST-MORTEM CASES: A 5 YEAR REVIEW**

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The Toxicology Unit, Imperial College London, undertakes Coroners' toxicology for cases in and around London. Mephedrone (4-methylmethcathinone) is a New Psychoactive Substance (NPS) commonly encountered in the UK. Whilst other NPS drugs seem to appear and disappear fairly rapidly; mephedrone has become established as a drug of choice and is regularly identified in our post-mortem samples.
Cases for a 5 year period (2010- March 2015) were retrospectively studied to identify mephedrone positive cases. A summary of toxicological findings from 60 mephedrone related deaths is presented.
Post-mortem femoral blood samples were routinely screened for a wide range of drugs using liquid-liquid extraction (LLE) followed by gas chromatography mass spectrometry (GC-MS) analysis. Mephedrone positive blood samples were quantified by GC-MS following LLE and N-methyl-bis (trifluoroacetamide)(MBTFA) derivatization.
The number of yearly cases and percentage distribution was as follows: 2010, 1 (0.11%); 2011, 5 (0.45%); 2012, 4 (0.30%); 2013, 16 (0.79%); 2014, 22 (1.00%); 2015 (first quarter), 12 (2.40%). Among these cases the mean age was 38 years (Range 21 - 71 Years) with the majority (92%) of the deaths being male. In 87% of the cases there was a history of previous drug use with drugs having been consumed directly prior to death in a third of the cases. Mephedrone was detected in blood samples with concentrations ranging from 0.05 – 7.0 μg/ml. In 95% of the mephedrone positive cases there was evidence of poly-drug use with between 1 and 5 other drugs and/or alcohol also being detected. In 83% of cases there were other 'club drugs' detected with classic amphetamines (3,4-methylenedioxy-metamfetamine (MDMA), amphetamine and metamfetamine) the most common type of drug being identified in 47% of cases. Other club drugs detected were gamma-hydroxybutyrate (GHB) - 33%, cocaine – 27%, ketamine – 7%, methiopropamine(MPA) – 3% and other NPS drugs including ethylphenidate, ethylone, methoxetamine and 6-(2-aminopropyl)benzofuran (6-APB) in one case each. Alcohol was measured in 28% of cases at concentrations ranging from 20 – 201 mg/dL. In addition, heroin use was identified in 12% of cases indicating mephedrone is not just used by typical club drug users.
Mephedrone is regularly identified in post-mortem cases and despite becoming a controlled drug in the UK (since April 2010) the frequency of cases is increasing. However, the number of deaths involving mephedrone (0.7% of total cases over the time period studied) is less than the number of deaths encountered where other classic stimulant drugs were detected: Amfetamine (1.8%), Methamfetamine (1.0%), MDMA (1.1%) or cocaine (7.5%). Mephedrone is rarely seen in isolation and is often taken in combination with other 'club drugs'. When carrying out toxicology analysis it is important to look for drugs that might not be routinely included in a general screen e.g. GHB or other NPS drugs.

MOP 20 DETECTION OF 31 STIMULANT, PSYCHEDELIC AND DISSOCIATIVE DESIGNER DRUGS IN REAL HAIR SAMPLES

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Many recreational drugs have been chemically synthesized in the last decade. Among them, the prevalent group (together with synthetic cannabinoids) is represented by synthetic cathinones, namely substituted phenethylamines compounds, with stimulant or psychedelic activity. Their abuse is documented across population groups from mid-to-late adolescents to older adults. However, this new class of substances is not routinely screened in most laboratories.

The detection of new psychoactive drugs in hair samples provides preliminary information about their current diffusion among the population and the typical sociological features of their users. Therefore, we developed and validated a new analytical method in order to detect the most common stimulant, psychedelic and dissociative new drugs in real hair samples.

A UHPLC-MS/MS method was developed to detect 26 stimulants and psychedelic substituted phenethylamines (3/4-MMC, 4-MEC, bk-MDMA, 4-FA, MDPV, pentedrone, ethylcathinone, α-PVP, bk-MBDB, buphedrone, 25I-NBOMe, 25C-NBOMe, 25H-NBOMe, 25B-NBOMe, 2C-P, 2C-B, 5-MAPB, 5/6-APB, PMMA, PMA, amfepramone, bupropion, mCPP and trazodone) and 5 dissociative drugs (MXE, PCP, 4-MeO-PCP, diphenidine, ketamine) in hair samples. Six deuterated internal standards (4-MMC-d3, MDPV-d8, MDMA-d5, 25I-NBOMe-d3, PCP-d5, m-CPP-d8) were used for the analytes quantitation. The method was fully validated and applied to 23 real samples taken from proven amphetamines and ketamine abusers (Group A). Furthermore, the method was applied to 54 real hair samples which had previously been tested negative within regular drug screening in driver's licence regranting (Group B).

The method proved to be simple, fast, specific and sensitive. The absence of matrix interferents, together with excellent repeatability of both retention times and relative abundances of diagnostic transitions, allowed the correct identification of all analytes tested. The method was linear in two different intervals at low and high concentration, with correlation coefficient values between 0.9981 and 0.9997. Quantitation limits ranged from 3.0 pg/mg for 4-MeO-PCP up to 57.8 pg/mg for 5/6-APB. The method was successfully applied to the analysis of real samples. In Group A, 5 samples tested positive for at least one analyte. MXE was found in 3 cases (range of concentration: 7.7-27.2 pg/mg); mephedrone (4-MMC) was found in 2 cases (50-59 pg/mg). Other sporadic findings included 4-MEC, α-PVP, methylone, 4-FA, MDPV and diphenidine. In group B, one sample tested positive to methylone at 27.2 pg/mg. All the remaining samples tested negative.

The elusive and changeable profile of the synthetic drugs progressively introduced into the black market makes any tentative study on their diffusion within our communities quite uncertain and incomplete. The use of hair analysis to investigate their diffusion among selected populations of drugs abusers may represent a practical tool to obtain significant information with limited investment, as the present study demonstrates. Furthermore, as long as this new class of substances will not be routinely screened in scheduled control programs (e.g. licence re-granting), an increasing risk exists that drug consumers will be induced to replace the traditional drugs of abuse with these new synthetic substances.

MOP 21 METHODS DEVELOPMENT FOR THE ASSESSMENT OF NPS USE BY ANALYSIS OF DIFFERENT BIOLOGICAL SAMPLES: HAIR, BLOOD AND URINE

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The identification of New Psychoactive Substances NPS in biological samples for the assessment of their use is of primary importance for forensic and clinical toxicologists. Depending on the finality of the analysis (treatment of intoxications and diagnosis of fatal intoxications, NPS-related addiction histories, evaluation of the spread of NPS among population), it may be necessary to analyze hair, urine and/or blood samples. High-throughput analytical methods, able to screen biological samples for this huge variety of analytes, similar to each-other, are therefore necessary.

The aim of the work was the development of multiresidual analytical methods able to identify in various biological matrices a wide number of NPS, including synthetic cannabinoids metabolites in urine, with high specificity and sensitivity.

Instrumental analyses were performed in UHPLC-MS/MS in ESI in MRM mode for the analysis of NPS. Analytes were grouped in three analytical methods with optimized elution gradients and MRM transitions: stimulants, synthetic cannabinoids, synthetic cannabinoids metabolites. Positive samples were submitted to further analyses in HRMS using a benchtop Orbitrap instrument at a resolving power of 100000 FWHM to confirm the accurate mass of the analyte, and subsequently by analysis in CID at a resolution of 50000 to study its characteristic fragments. Samples preparation: Hair samples (30 mg) were incubated in aqueous formic acid overnight for the extraction of Amphetamine-Type Stimulants (ATS), cathinones, ketamine and analogues, piperazines and other stimulants; synthetic cannabinoids were extracted by a further incubation in methanol overnight. The extracts were directly injected in the UHPLC-MS/MS system. Blood samples were purified by a Dispersive Liquid/Liquid MicroExtraction (DLLME) after deproteinization with methanol. Urine samples were diluted 1:5 and directly analyzed in the LC-MS/MS system. For the screening of synthetic cannabinoids metabolites a method in precursor ion mode was developed, able to screen for the common fragments of the main metabolites of synthetic cannabinoids.

The methods developed allowed the screening of more than 70 NPS with a satisfactory sensitivity. All the methods

showed good linearity and accuracy. Recoveries were above 80% for synthetic cannabinoids and ATS in authentic hair samples; for blood samples, DLLME gave recoveries from 10 % (synthetic cannabinoids) to 99% The methods were then applied to authentic specimens demonstrating their suitability for the identification of NPS of various classes in biological specimens. In four hair samples we detected synthetic cannabinoids, while cathinones and ephedrines were detected in seven samples; in 5 hair samples it was identified ketamine and NM2AI (N-methyl 2 aminoindane) in two hair samples. In two blood samples we detected ephedrines, in one samples a mixture cathinones and NM2AI. We detected ketamine in ten urine samples, m-chlorophenylpyperazine in three samples, while no synthethic cannabinoids were detected neither in blood nor in urine samples.
High-throughput LC-MS/MS methods were set up for the screening of more than 70 NPS of different classes in three kinds of biological specimens: hair, urine and whole blood, useful for the assessment of chronic/acute intoxications or identification of the use of these substances in epidemiological studies.

4.30-6.00pm SESSION 4 - Toxicokinetics II

MOP 22 METABOLISM OF MAJOR PHYTOCANNABINOIDS BY HUMAN PLACENTA MICROSOMES AND CYP19

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Major phytocannabinoids (Δ9-tetrahydrocannabinol, Δ9-THC; cannabidiol, CBD and cannabinol, CBN) have been reported to be extensively metabolized by human hepatic microsomal cytochrome P450 (CYP). However, relatively little is known about their metabolism by extra-hepatic CYPs (brain and lung etc.). The intake of cannabis has been linked to the adverse effects on reproductive function and fetal development. The metabolism of cannabinoids is one of the important factors affecting their levels in the fetal circulation. In the present study, we examined the metabolism of three major phytocannabinoids by human placenta microsomes (HPMs) and recombinant human CYP19.
To clarify metabolism of major phytocannabinoids by extra-hepatic cytochrome P450 (placenta microsomes and CYP19)
Human placenta and recombinant human CYP19 were obtained from Veritas Corp. and BD Gentist, respectively. HPMs were prepared by the established method.Δ9-THC, CBD and CBN were incubated with HPMs or CYP19 in the presence of an NADPH-generating system. The metabolites formed were extracted with ethyl acetate and analyzed with their typical diagnostic ions by GC-MS after trimethylsilylation.
Δ9-THC was exclusively metabolized to 8α-hydroxy-Δ9-THC by HPMs and CYP19. CBD was metabolized to 6α-, 6β- and 7-hydroxy-CBD. Among them, 6α-hydroxy-CBD was the most abundant metabolite. CBN was metabolized to 8- and 11-hydroxy-CBN, in which 8-hydroxy-CBN was a more abundant metabolite by two enzyme sources. The formation of 8α-hydroxy-Δ9-THC from Δ9-THC was markedly inhibited (~ 90%) by androstenedione (AD) and aminoglutethimide (AG)(10 μM), a substrate and an inhibitor of CYP19, respectively. The formation of 8- and 11-hydroxy-CBN from CBN were inhibited by 35~65% of the control with AD and AG. AD and AG did not potently inhibit the metabolite formation (~ 35%) from CBD by HPMs and CYP19.
The present study demonstrated that HPMs and CYP19 are able to metabolize three major phytocannabinoids mainly at the 8- (Δ9-THC and CBN) and 6-position (CBD). Keywords. Cannabidiol, Cannabinol, Δ9-Tetrahydrocannabinol, Metabolism, CYP19, Placenta microsomes.

MOP 23 METABOLISM OF 25I-NBOME AND 25C-NBOME, TWO SYNTHETIC HALLUCINOGENS, IN MICE AND CORRELATION WITH AUTHENTIC HUMAN URINE SAMPLES

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25I-NBOMe and 25C-NBOMe, two synthetic hallucinogens, are N-2-methoxybenzyl derivatives of the psychedelic compounds 2C-I and 2C-C and emerged recently as new psychoactive substances. Both are super potent 5-HT2A receptor agonists sold as LSD alternatives. 25I-NBOMe is the most popular member of the NBOMe series and was linked to a number of fatalities. Several European countries, Russia and the US scheduled NBOMe substances.
Our goal was to perform a comprehensive metabolism study as no data on these relatively new recreational drugs are currently available.
We administered 1mg/kg 25I-NBOMe and 25C-NBOMe to C57BL/6N mice and collected urine over 24 h. Additionally, an authentic human urine specimen for each compound was analyzed. Samples were diluted with mobile phase directly or after hydrolysis with beta-glucuronidase/sulfatase and analyzed on an Agilent LC-QTOF G6550. LC conditions were as follows: gradient elution over 12 min; column, HSS T3 (2.1*150 mm); mobile phase A, 0.05% formic acid in 10 mM ammonium formate; mobile phase B, 0.05% formic acid in acetonitrile; flow rate 0.5 mL/min. To identify possible metabolites, samples were analyzed in data-dependent mode with mass range m/z 100-1000 and a threshold of >5000 cps in positive ESI. Accurate full scan MS and MS/MS data were analyzed in two ways: 1) manually by detecting metabolites generated by common metabolic reactions and 2) software-assisted by WebMetabase (Molecular Discovery). Preliminary results were used to generate a preferred list of possible metabolites, and samples were injected again to produce high quality MS/MS spectra. All metabolites were evaluated for mass error (≤5 ppm), conclusive isotopic and fragmentation pattern and plausible retention time and were compared to

in silico predictions generated by Metasite (Molecular Discovery).
In mice, we identified 18 25I-NBOMe metabolites, generated predominantly by demethylation, di-demethylation, hydroxylation and combinations; many phase I metabolites were further glucuronidated. In the human urine specimen, 14 25I-NBOMe metabolites were found, all previously identified in mouse urine, and the same metabolic reactions were observed, although relative intensities slightly differed. Only glucuronides were observed as phase II metabolites. Parent 25I-NBOMe was detected, but at a low intensity. For 25C-NBOMe, we identified 17 metabolites in mice, the majority generated by demethylation, di-demethylation and hydroxylation. In contrast to 25I-NBOMe, parent 25C-NBOMe ranked #1 or 2 in terms of intensity in the non-hydrolyzed and hydrolyzed sample, respectively. Glucuronidation was the most dominant phase II reaction, but also sulfation and acetylation occurred. In human urine, almost all mouse urine metabolites were observed in similar intensities. As previously shown, both parents are stable in urine under different conditions. MetaSite correctly predicted the most intense metabolites in human urine specimens.
There was good agreement between mouse and human metabolism. The metabolic stability of the 25I-NBOMe was much lower than of 25C-NBOMe. To prove intake, we recommend that human urine samples are hydrolyzed and that 25I-NBOMe metabolites demethylated at the 2'-position of the phenyl ring and hydroxylated at the NBOMe ring are targeted. For documenting 25C-NBOMe intake, the parent compound is a good analytical target as well as the demethylated and di-demethylated metabolites.

MOP 24 IN VITRO METABOLISM STUDIES AND LC-MS CHARACTERIZATION OF SARM LG121071 FOR ROUTINE DOPING CONTROL

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Selective androgen receptor modulators (SARMs) like the tetrahydroquinolinone derived LG121071 represent a class of nonsteroidal therapeutics considered for hormone-replacement therapy in androgen deficiency diseases. These agents offer anabolic effects like muscle growth and increase of bone density as well as increased physical strength and fitness. In contrast to anabolic steroids their oral bioavailability is improved with less adverse androgen cross reactivity and provide strong incentive for doping abuse in sports. Even though they are not clinically approved yet, SARMs are an emerging class of drugs on the black-market and several SARM-positive doping control samples have been reported since 2010.
The aim of the present study was to investigate the metabolic fate of SARM LG121071 by in vitro simulation and the identification in human urine doping control samples by high resolution/high accuracy LC-MS(/MS) analysis in electrospray ionization positive mode.
For in vitro metabolism studies LG121071 was incubated with human liver microsomes and established co-substrates in phosphate buffer pH 7.4. For detection of analytes and further structure elucidation, a gradient elution was performed by a Nucleodur Pyramid C18 analytical column followed by TOF-MS and Orbitrap-MS detection, respectively. Sample preparation procedure for validation, with 17-α methyltestosterone as internal standard, included enzymatic hydrolysis of glucuronides prior to liquid/liquid extraction and LC-MS(/MS) detection.
Although the overall extent of metabolism was modest, mono-, bis- and tris-hydroxylated metabolites as well as glucuronidation of the active substance were observed in the in vitro experiments. For that reason the parent was expected to represent the diagnostic molecule. Its dissociation pathway was elucidated and diagnostic product ions are m/z 267.0740, m/z 253.0583 and m/z 241.0583. The lower limit of quantification was determined at 0.5 ng/mL and the recovery was 40±9%. Intra- and interday precisions were investigated at low (1 ng/mL), medium (10 ng/mL) and high (200 ng/mL) concentrations ranging from 2.3 to 11.7%.
The presented LC-MS(/MS) detection method aiming at the intact substance is considered a viable option for routine doping controls and is fully validated. The intact SARM LG121071 is suggested to represent a sensible target analyte, especially since its glucuronide is susceptible of enzymatic hydrolysis.

MOP 25 INHIBITION POTENTIAL OF MDMA AND ITS RELEVANT METABOLITES ON THE IN VITRO DEAMINATION OF SEROTONIN AND DOPAMINE

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Neurotoxicity of 3,4-methylenedioxymethamphetamine (MDMA) is still controversially discussed. MDMA metabolism seems to play a major role in the observed neurotoxic effects, as its direct injection into the brain failed to reproduce the neurotoxic damage. Previous studies have shown an inhibition potential of MDMA metabolites regarding the degradation of the neurotransmitter dopamine (DA) by catechol-O-methyltransferase and sulfotransferase (Meyer et al, Chem Res Toxicol, 2009; Schwaninger et al, Toxicol Lett, 2011). Their influence on the major dopamine degradation pathway by monoamine oxidase (MAO) in humans has not yet been studied.
The aim of the present work was to evaluate the inhibition potential of MDMA and its relevant metabolites regarding the main degradation pathway of the neurotransmitters DA and serotonin (5-HT) by MAO A and B using recombinant human enzymes in vitro.
Initial in vitro experiments were performed with recombinant human MAO A and B (0.0125 mg/mL), DA (100, 150 µM) and 5-HT (100, 500 µM) and two different concentrations (2.5, 50 µM) of the potential inhibitors MDMA, 3,4-methylenedioxyamphetamine (MDA), 4-hydroxy-3-methoxyamphetamine (HMA), 3,4-dihydroxymethamphetamine

(DHMA), 4-hydroxy-3-methoxymethamphetamine (HMMA) and their respective sulfate and glucuronide conjugates (n=5). Selegiline and moclobemide were used as positive controls (n=5). For determination of the underlying inhibition model, Michaelis-Menten kinetics were performed with DA and 5-HT concentrations ranging from 10-1000 µM and inhibitor concentrations ranging from 0 to 50 µM (n=2). Lineweaver-Burk and Dixon plots were plotted using GraphPad Prism 6.02. After 15 min, the incubation reactions were stopped with acetonitrile and the supernatants were analyzed after centrifugation and dilution by LC-MS/MS (ABSciex 5500 Qtrap; Dionex UltiMate 3000; ZIC-HILIC column (150 x 2.1 mm I.D., 3.5 µm)).
Based on the initial experiments MDMA and MDA showed relevant inhibition (> 30%) towards MAO A for DA and 5-HT. No relevant effects towards MAO B were observed. Further investigation of the underlying inhibition model revealed the best fit for a competitive inhibition of MDMA on DA and 5-HT deamination with Ki of 18.6 +- 4.3 µM (MDMA, DA, MAO A) and 24.5 +- 7.1 µM (MDMA, 5-HT, MAO A). Inhibition by MDA was best described applying a mixed-type inhibition model with Ki values of 8.4 +- 3.2 µM (MDA, DA, MAO A) and 7.8 +- 2.6 µM (MDA, 5-HT, MAO A). Estimation of the in vivo relevance as proposed by Bachmann (Drug Metabol Disp, 2006) resulted in inhibitor concentration/Ki ratios of 0.11 (MDMA, DA), 0.08 (MDMA, 5-HT), and 0.01 (MDA, DA and 5-HT).
MDMA and MDA were shown to act as competitive and mixed-type inhibitors of in vitro MAO A deamination of DA and 5-HT, most probably due to their structural similarities to the neurotransmitters with slightly higher inhibition potential of MDA. However, estimation of the in vivo relevance based on the calculated Ki values indicates only a medium risk for a relevant inhibition of DA and/or 5-HT deamination in vivo.

MOP 26 PHARMACOKINETIC MODELLING OF JWH-210, RCS-4, AND THC IN PIG SERUM AFTER INTRAVENOUS ADMINISTRATION

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For a few years, synthetic cannabinoids have been increasingly used as drugs of abuse. In clinical toxicology as well as in forensic expert opinion, detailed knowledge of pharmacodynamic and pharmacokinetic properties may be of great importance. As controlled clinical trials with new drugs of abuse cannot be conducted for ethical reasons, animal studies are the best way to obtain pharmacokinetic data as those from case work are incomplete.
The first aim was to develop a pharmacokinetic model of the two synthetic cannabinoids JWH-210 and RCS-4 in comparison to THC in pigs. The second aim was to assess whether the pharmacokinetic data for THC measured in pigs can be correlated with published data in humans.
All experiments were approved by the governmental Committee on Animal Affairs. Isoflurane-anaesthetized domestic pigs (n = 6 per drug; mean body weight 45.2 ± 7.3 kg) received a single 200 µg per kg body weight dose of JWH-210, RCS-4, or THC into the jugular vein. Blood samples were drawn before and 1, 2, 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, 300, and 360 min after administration. The serum concentrations were determined with a validated LC-MS/MS method in positive APCI mode after SPE (Schaefer et al., ABC, 2015). Non-linear mixed effects modelling approach was applied using NONMEM 7.3 (ICON development solutions, San Antonio, USA). Several structural models were tested. Model selection was based on the visual inspection of goodness-of-fit plots, precision of parameter estimates and the statistical values provided by NONMEM. The final THC model in pigs was upscaled to humans using allometric principles and the human predicted exposure was compared to published concentration-time profiles.
Mean maximum concentrations (1 min after administration) were 1,600 ± 362 ng/mL for JWH-210, 1,438 ± 346 ng/mL for THC, and 316 ± 60 ng/mL for RCS-4 and decreased to mean concentrations of 3.1 ± 1.1 ng/mL for JWH-210, 7.1 ± 4.3 ng/mL for THC, and 3.8 ± 1.1 ng/mL for RCS-4 after 360 min. The serum-concentration-time profiles of JWH-210, RCS-4, and THC were best described by 3-compartment models with first order elimination processes. All parameters were estimated precisely. Volumes of distributions at steady-state were estimated at 67.6 L, 194 L, and 917 L for JWH-210, RCS-4, and THC, indicating a distribution into deeper compartments. Systemic clearances were estimated at 48.7 L/h, 83.4 L/h, and 7.0 L/h for JWH-210, RCS-4, and THC. The allometrically scaled pharmacokinetic model in pigs for THC predicted published concentration-time-profiles in humans after intravenous administration very well.
Three pharmacokinetic models were successfully developed describing the serum-concentration-time profiles of JWH-210, RCS-4, and THC after intravenous administration in domestic pigs. The successful THC prediction of human exposure based on the domestic pig pharmacokinetic model suggest that domestic pigs in conjunction with pharmacokinetic modelling technique may serve as a tool for the future prediction of human exposure of synthetic cannabinoids.

MOP 27 THE USE OF CYTOCHROME P450 2D6 AND 2C19 GENOTYPING FOR TOXICOLOGICAL INTERPRETATION OF SPECIAL FORENSIC CASES – USEFUL OR NOT?

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In some drug poisoning cases it is not clear what caused high drug concentrations or why certain metabolites are lacking. It could be speculated that this was caused by reduced elimination capacity. The polymorphic cytochrome P450 (CYP) isoforms 2D6 and 2C19 are the major metabolizing enzymes for a number of drugs. Thus, the post mortem finding of high drug concentrations or missing metabolites could be explained by poor metabolizer (PM) status of CYP2D6 or CYP2C19.
To discuss the value of information obtained by CYP2D6/2C19 genotyping in four poisoning cases.
Screening and quantification was done using several techniques for different compound classes, among these LC-TOF-MS for screening and LC-MS-MS for quantification. Sample preparation was performed by protein precipitation using deuterated internal standards. Genotyping for CYP2C9*2,*3,*4,*5; CYP2C19*2,*3,*4,*17; CYP2D6*3,*4,*5,*6 and CYP2D6 gene copy number was performed using PCR and Pyrosequencing at Department of Forensic Genetics and Forensic Toxicology, Linköping, Sweden.
Case 1: Male, 35 years, assessed to be a fatal combined poisoning by venlafaxine 4.5 mg/kg, ethanol 1.2 g/kg, and oxycodone 0.28 mg/kg. PM of both CYP2D6(*4,*5) and CYP2C19(*2,*2). It was concluded that the combined PM status was the cause of the high venlafaxine concentration. Case 2: Female, 75 years, tramadol administration error at the hospital causing a fatal serotonergic syndrome due to selegiline (0.09 mg/kg) and tramadol (1.1 mg/kg) interaction. No significant O-desmethyltramadol was found (tramadol metabolite mainly formed by CYP2D6). There were no other drug-interactions. Extensive metabolizer (EM) of CYP2D6(*1,*4) confirmed that the tramadol dose most likely have been taken close to time of death and that the high tramadol concentration was not caused by missing CYP2D6 activity. Case 3: Male, 47 years, considered to be a fatal poisoning by nortriptyline 2.9 mg/kg. Nortriptyline metabolism is highly dependent on CYP2D6. EM of both CYP2D6(*1,*4) and CYP2C19(*1,*1) was established. It was concluded that the high nortriptyline concentration had other causes than missing CYP2D6 activity. Case 4: Female, 50 years, died despite intensive resuscitation High concentration of venlafaxine 4.2 mg/kg and O-desmethylvenlfaxine 3.0 mg/kg was found. EM of both CYP2D6(*1,*1) and CYP2C19(*1,*2) was established. The high concentrations of venlafaxine and O-desmethylvenlfaxine were not explained by missing enzyme activity, and could have been an artifact introduced by the intensive resuscitation.
Genotyping was considered beneficial in these special forensic cases. It either gave an explanation for the high drug concentrations, or gave more information to whether the cause of death was an accidental or intentional poisoning. We only recommend to genotype in special forensic cases, where the pathways of drug metabolism and case story supports this.

MOP 28 ENANTIOSELECTIVE PHARMACOKINETICS OF TRAMADOL AND ITS THREE MAIN METABOLITES; IMPACT OF CYP2D6 AND CYP2B6 GENOTYPE

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The main cytochrome P450 (CYP) enzymes responsible for the metabolism of the chiral drug tramadol are CYP2D6, CYP2B6 and CYP3A4. The first-mentioned is involved in the formation of O-desmethyltramadol (ODT) and NO-didesmethyltramadol (NODT) while the other two produce N-desmethyltramadol (NDT) and NODT. It has earlier been shown that the CYP2D6 genotype influence the amount of the pharmacologically active ODT, with poor metabolizers having significantly lower concentrations compared to extensive metabolizers. The (+)-enantiomer of ODT, exerting most of the opioid effects, is affected to a larger extent than the (-)-enantiomer. The enantiomer concentrations of tramadol and all three main metabolites have however not been simultaneously determined earlier. Neither has the potential impact of the CYP2B6 genotype on the pharmacokinetics of tramadol been investigated.
The purpose of the study was to investigate interindividual differences in tramadol pharmacokinetics by enantioselective analysis of tramadol and its three main metabolites in relation to CYP2D6 and CYP2B6 genotype.
Nineteen healthy volunteers were recruited and randomized into two groups receiving a single dose of either 50 or 100 mg of orally administrated tramadol. Blood samples were collected at 17 occasions; prior to dosing and up to 72 hours after drug administration. The enantioselective analysis of tramadol, ODT, NDT and NODT in whole blood was performed with a validated LCMSMS-method in reversed phase mode using a chiral AGP (α1-acid glycoprotein) column. The limit of quantification (LOQ) was 0.125 ng/g for all enantiomers except for (+)- and (-)-ODT with a corresponding limit of 0.50 ng/g. Pyrosequencing technology was utilized for CYP2D6 and CYP2B6 (516 G>T, 785 A>G, 1459 C>T) genotyping.
Currently, 14 individuals have been analyzed. Their CYP2D6 genotypes corresponded to two extensive metabolizers, four intermediate metabolizers and one poor metabolizer in each dosage group. The poor metabolizers, both with CYP2B6 wild type alleles, showed an enantioselective metabolite pattern different from extensive and intermediate metabolizers. Most apparent was the considerably lower ratios of (+)-ODT/(-)-ODT and (+)-NODT/(-)-NODT. The individual administrated 50 mg never presented with (+)-ODT or (+)-NODT levels above LOQ. Seven individuals had at least one of the investigated CYP2B6 polymorphisms. One of them, being homozygous for the 1459 C>T polymorphism showed lower concentrations of both the (+)- and (-)-enantiomer of NDT and NODT, most obvious in comparison to an individual with wild type CYP2B6 alleles but with the same CYP2D6 genotype (corresponding to an extensive metabolizer) and being administrated the same dose (100 mg).
These preliminary data suggests that CYP2D6 poor metabolizers have low enantiomeric ratios of both ODT and NODT, consistent with the CYP2D6 enzyme being stereoselective. Homozygosity for the CYP2B6 polymorphism 1459 C>T indicated a reduced enzyme function with lower concentrations of NDT and NODT. This finding must however be confirmed and the other polymorphisms further investigated, preferably in a larger sample.

8.30-10.30 am SESSION 1 – Driving Under the Influence I

TuOP 1 EVALUATION OF DELTA-9-TETRAHYDROCANNABINOL CONCENTRATIONS IN DUID INVESTIGATION CASES AND CONSEQUENCES OF VARIOUS THC PER SE THRESHOLDS.

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Many of US states are moving forward to legalize recreational marijuana use and to establish THC per se law.
To obtain statistical information on blood THC concentrations and identify the pattern of concomitant drug use in DUID investigations. Also, to understand the consequence of the per se concentration thresholds.
From August 2009 to October 2014, a total of 36,037 DUID cases were submitted to NMS Labs from various law enforcement agencies throughout the United States. Of these, 48.8% (n=17,612) screened positive for the presence of cannabinoids by ELISA and further analyzed by multi-dimensional Gas Chromatography/Mass Spectrometry (GC-GC-GC/MS) to quantitate Delta-9 THC, THC-COOH and THC-OH.
A total of 13,988 (79.4%) cases were confirmed positive for at least one measured cannabinoid compound (Delta-9 THC, THC-COOH and THC-OH). When gender and age were provided, 80.6% of the drivers were male. The mean and median ages of the drivers were 29 and 25 years old, respectively (range; 15-75). Of confirmed cannabinoid positive cases, 53.2% (n=7,448) were also tested for alcohol and common drugs of abuse. The cases were divided into the following four groups: A. positive for THC with other drugs and alcohol (n=1,159, 15.6%); B. positive for THC without other drugs but with alcohol (n=3,178, 42.7%); C. positive for THC with other drugs detected but no alcohol (n=1,279, 17.1%); and D. positive for THC only (n=1,832, 24.6%). Ethanol above 0.01% BAC were present in 4,337 (58.2%) cases (Group A and B). Of the 2,438 (32.7%) cases presumptively positive for the presence of other drugs (Group A and C), opiates (18.3%) was the most prevalent, followed by benzodiazepines (16.0%), cocaine (8.49%) and amphetamines (6.42%). Among the confirmed cannabinoid positive cases, the mean, median and range of blood THC concentrations as well as frequency of Delta-9 THC above the reporting limit of 1.0 ng/mL were determined: 5.9 ng/mL, 3.8 ng/mL, 1-99 ng/mL and 80.4%. The results for each group were also examined (mean, median and range (all in ng/mL), frequency (in %): A. (4.3, 2.8, 1-45, 72.5%); B. (4.7, 3.2, 1-47, 79.0%); C. (6.2, 3.9, 1-99, 75.2%); and D. (8.2, 6.0, 1-84, 91.5%). To assess the consequences of various THC per se thresholds, we determined percentage of DUID cases where cannabinoids were screened positive but confirmatory testing resulted in None Detected for Delta-9 THC based on the per se law. When all groups were combined, approximately 20, 40 and 70 % of presumptively positive cases were below blood THC concentrations of 1.0 ng/mL, 2.0 ng/mL and 5.0 ng/mL, respectively. Group A had approximately 81% of the population with Delta-9 THC concentration less than 5.0 ng/mL.
With higher THC per se laws, a large population of drivers suspected of DUID will have blood THC concentrations below the thresholds despite the presence of signs and symptoms consistent with marijuana impairment. Because Delta-9 THC rapidly dissipates from the bloodstream and redistributes away from blood, as per se concentrations increase, it becomes more difficult to corroborate lab THC results with on scene observations of THC impairment.

TuOP 2 GHB IN SUSPECTED IMPAIRED DRIVERS IN THE NETHERLANDS AND A COMPARISON WITH POSTMORTEM CASES.

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In the Netherlands, GHB is a frequently used and misused drug. Information about the prevalence of GHB in drivers and the number of fatalities involving GHB is limited.
Aim of this study was to investigate the prevalence of GHB in suspected impaired drivers (DUI cases) and postmortem cases. In addition, the GHB concentrations in postmortem cases were compared with concentrations in non-fatal DUI-cases to get more insight in the overlap between recreational concentrations and concentrations found in GHB related or caused deaths.
The prevalence of GHB in suspected impaired drivers was investigated by reviewing the results of the blood samples which were sent to the Netherlands Forensic Institute (NFI) for toxicological analysis during the years 2009-2012. Postmortem cases were selected in which an autopsy was performed by the pathologists of the NFI during the same period and in which the presence of GHB in femoral blood was demonstrated at a concentration higher than 50 mg/L and in which recent exposure to GHB was supported by the concentration of GHB in other human matrices. GHB was determined by using gas chromatography with mass spectrometric detection. The limit of quantification was 5 mg/L.
GHB was demonstrated in 22% (669/3038) of the blood samples of suspected impaired drivers. In 5.9% (179/3038) of the cases, no other illicit drug was detected. The most frequently detected combinations of GHB and other illicit drugs in drivers were GHB and amphetamines 9.7% (295/3038), GHB and cocaine 2.5% (75/3038), GHB, amphetamines and cocaine 1.4% (41/3038), GHB, amphetamines and cannabis 1.1% (33/3038), GHB and cannabis 1.0% (29/3038). Alcohol was not included in the review of the DUI cases because the results of the alcohol breath test (if performed) were unknown. The GHB concentrations in DUI cases ranged from 5.2 to 924 mg/L (median 94 mg/L). Exposure to GHB was concluded in only 1.9% (21/1105) of the postmortem cases. The GHB concentrations in drug related and drug caused deaths ranged from 60 to 932 mg/L (median 191 mg/L).

In the Netherlands, exposure to GHB was concluded in 22% of the suspected impaired drivers and in 1.9% of the postmortem cases during the period 2009-2012. Frequently, GHB was taken in combination with other illicit drugs. The GHB concentrations in DUI cases overlap the range of concentrations in postmortem cases. Therefore, in addition to the toxicological results, circumstances and pathology findings are needed to establish the cause of death.

TuOP 3 PREVALENCE OF NEW PSYCHOACTIVE SUBSTANCES IN DRUG DRIVERS IN SCOTLAND - A 2 YEAR REVIEW

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Scottish Police Authority (SPA) Forensic Services is the specialist laboratory that analyses samples in Scotland obtained for the purposes of Section 4 (DUID) of the Road Traffic Act, 1988. SPA receives approximately 300 Section 4 blood and urine samples annually for screening to identify the presence of drugs capable of impairment. The number of Section 4 cases positive for New Psychoactive Substances (NPS) in 2013 and 2014 has been evaluated and the results assessed.
The aim of the study was to identify any New Psychoactive Substances present in drivers in Scotland during 2013 and 2014 and to identify any apparent trends in drug use amongst the population.
Blood and urine samples submitted for the purposes of Section 4 of the Road Traffic Act were screened for alcohol (GC-HS), drugs of abuse (ELISA, Triple Quad LCMS) and NPS(Triple Quad LCMS)as a minimum. Initially, approximately 40 different compounds were screened for using a targetted screen. However, this has evolved as the drug trends in Scotland have changed and the most recent method includes approximately 80 different NPS. All results obtained were collated and those that were positive for a NPS were further interrogated. Drug types assessed include cathinones, piperazines, synthetic cannabinoids, phenethylamines, new generation benzodiazepines and a miscellaneous group of drugs that do not easily fall into one of those named. The positive cases were assessed to identify any trends in drug use among the drug driving population in Scotland.
In 2013 and 2014, 26% and 27% respectively of cases submitted under Section 4 of the Road Traffic Act were positive for at least one NPS. The large majority of NPS positive cases were for new generation benzodiazepines such as phenazepam (46%), etizolam (28%) and diclazepam (4.4%). Other prevalent drugs include mephedrone (11%), MPA (4.4%) and ketamine (2.5%). NPS detected to a lesser degree included GHB, BZP, APB and methylone. Except for synthetic cannabinoids (<1% of cases positive), the drugs detected are consistent with the trends experienced in NPS seizures by Police Scotland.
Drivers suspected of being impaired through the use of drugs in Scotland have demonstrated a high prevalence of NPS use. In particular, new generation benzodiazepines such as phenazepam and etizolam are regularly detected.

TuOP 4 CRASH RISK OF MEDICINAL DRUGS

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Road trauma involving drugs is a recognized public health issue world-wide. Crash risk associated with drugs such as alcohol and many of the drugs of abuse are reasonably well established; however there is relatively little data on the crash risk associated with medicinal drugs that are psychoactive, such as the benzodiazepines, opiates/opioids, antidepressants, sedating antihistamines and the antipsychotic drugs.
To determine the incidence of alcohol and drugs in Victorian fatally-injured drivers with particular focus on medicinal drugs that may have the ability to impair driving.
Driving circumstances and toxicology results on driver fatalities in Victoria were obtained from the National Coroners Information System (NCIS) and information held at the Coroners Court of Victoria and the Victorian Institute of Forensic Medicine over a 14-year period from 2000 to 2013. Each driver was assessed for culpability using a previously published responsibility analysis method. Crash risk was calculated using the culpability ratios of drug groups. Odd's Ratios (OR) was determined using the drug free driver as control.
2638 drivers were included in the study of which 54% were positive to alcohol and/or drugs. Alcohol (0.05% or higher) was present in 24% of drivers and illicit drugs were present in 19% of drivers, while drugs of any type were found in 42%. The most common medicinal drug types were (in order of prevalence); antidepressants (10.3%), benzodiazepines (8.3%), opiates/opioids (7.2%), while antipsychotics and sedating antihistamines were both present in 2.0% of drivers. Substantial numbers of drivers were positive to more than one substance capable of causing impairment. Culpability analysis showed no significant over-representation of drivers using medicinal drugs such as opiates/opioids, benzodiazepines, antidepressants, antipsychotics or antihistamines, although all of these showed some increase over unity suggesting that these drugs may have a small effect. In contrast, drivers using alcohol ≥0.05% (OR=8.6), any psychoactive drug only (OR 2.0), THC only (OR=2.4) and amphetamine-type stimulants only (OR 4.1) were significantly over-represented (P<0.05).
Medicinal drugs do not exhibit measurable increases in crash risk when compared to the increase in crash risk associated with illicit drugs and combinations of psychoactive drugs.

TuOP 5 **DRIVING UNDER THE INFLUENCE OF MARIJUANA VERSUS DRIVING AND DYING UNDER THE INFLUENCE OF MARIJUANA: A COMPARISON OF BLOOD CANNABINOID CONCENTRATIONS IN ARRESTED DRIVERS VERSUS DECEASED DRIVERS**

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Cannabis intoxication is an important medico-legal topic. Several jurisdictions have blood THC per se DUI limits. In California such legislation has not been yet enacted. There are only a few studies on cannabinoids in living or deceased humans but they suggest changes occur due to PMR and PMI. Notwithstanding, postmortem toxicology reports are, unfortunately, still used to infer antemortem intoxication or impairment.
The study ascertains blood cannabinoids in living and deceased drivers and evaluates Huestis' Models I & II and 95% Confidence Intervals (CIs) in 23 living drivers with known time points of driving, arrest and blood draw.
Four years (2010-2013) of cannabinoid concentrations in two groups of drivers were analyzed. Group 1 comprised of 318 living drivers arrested and found to have cannabinoids in their blood. Group 2 comprised of 23 drivers who died while operating a vehicle and found to have cannabinoids in their postmortem peripheral blood. An ABFT-accredited lab employed ELISA (cutoff = 5 ng/mL) and LC-MS/MS after LLE with LOQ of 1 ng/mL for THC, THC-OH, CBD and CBN and LOQ of 5 ng/mL for THC-COOH to perform all measurements in ng/mL [mean; median].
Group 1 had THC, THC-OH and THC-COOH of [4.9; 3], [4.7; 3], and [64.0; 41]. A subgroup of Group 1 that comprised of 88 subjects with only cannabinoids found in their blood, had THC, THC-OH and THC-COOH of [5.8; 4], [4.6; 3], and [77.1; 50.5]. In 19 of Group 2's subjects, cannabinoid measurements were performed in peripheral blood. Their THC, THC-OH and THC-COOH were [11.7; 4.5], [7.7; 4.5], and [79.2; 41]. In 9 of them, cannabinoids were the only psychoactive compounds detected and in 8 of those, measurements were performed in peripheral blood. In those 8 cases, THC, THC-OH and THC-COOH were [20.3; 19.5], [11.2; 4]; and [114.7; 44]. Motorcyclists and bicyclists comprised the largest two subgroups of Group 2 and bicyclists had the highest peripheral blood [THC] (mean: 31.3; median: 24 ng/mL). ANOVA showed that THC-OH and THC-COOH concentrations were not statistically different between Group 1 and 2. The THC concentrations between Group 1 and 2, however, showed statistically-significant differences. In a subgroup of Group 1, the Huestis' Models and their 95% CIs were calculated. The Models predicted elapsed times after marijuana use consistent with the case's actual time points in 70% of cases but once the 95% CIs were determined, the resulting prediction ranges were consistent with the case's actual time points in all 23 cases (100%).
This study offers cannabinoid concentration ranges in living and deceased drivers and shows that operators of two-wheeled vehicles comprise the largest portion of cannabinoid-positive deceased drivers. It also shows that it is scientifically unwise to compare postmortem [THC] to clinical concentrations or to evaluate postmortem [THC] based on per se laws applicable to living subjects. THC-OH and THC-COOH blood concentrations appear, yet again, to be better compounds for such comparisons or evaluations. Finally, in a subgroup of 23 cases, the Huestis' Models and 95% CIs were 100% successful in their predictions.

TuOP 6 **DRIVING UNDER THE INFLUENCE OF ALCOHOL AMONG CAR DRIVERS IN ISRAEL**

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Alcohol is one of the main causes of traffic accidents worldwide. In accordance with the Israeli law, subjects are considered to be positive when blood alcohol concentration (BAC) exceeds 50 mg/100 mL, except for special populations (drivers under 24 year old, newly licensed drivers and public/heavy transportation drivers) for which a "zero tolerance" (5 mg/100 mL) approach applies. Although the "zero tolerance" law was approved several years ago, its enforcement started on 2014.
The aim of the present study was to assess the blood alcohol concentration of Israeli drivers by age, gender, driving offense and severity of car accidents between 2012 and 2014 and the impact of the "zero tolerance" law on the data distribution and on detection of traffic offenses in the "special population".
A retrospective analysis of 1581 individuals stopped by traffic police because of erratic driving or involvement in car accident and tested for BAC between 2012 and 2014 was performed. Relations between BAC, demographic parameters, type and severity of driving offense within the observed time frame were analyzed. BAC was measured by headspace gas chromatography with flame ionization detection at the Clinical Pharmacology and Toxicology Laboratory, Sheba Medical Center (Israel).
Analyses of demographic parameters showed that approx. 90% of the individuals tested for BAC were males and that the number of drivers below 24 years old tested for BAC increased from 16.1% in 2012 to 39.8% in 2014. Accordingly, the number of young drivers tested that were involved in car traffic accidents increased from 16% in 2012 to 27.8% in 2014 and in other traffic offenses (driving under the influence, driving without driving license, etc.) increased from 15.2% to 60.1%, respectively. Alcohol was detected in the blood of about 70% of all the traffic -related cases during 2012 to 2014. The number of young drivers tested positive at a BAC ≤50 mg/100mL increased 2.8 fold from 2012 to 2014. Nevertheless, 56% of the subjects involved in all types of traffic accidents were younger than 35 year old. In 83.4% of the fatal car accidents, BAC was not detected. However, 41.1% and 57.4% of the individuals involved in severe and minor accidents respectively ranged between 50 to 200 mg/100 mL.
Our results reflect the enforcement extent of the zero tolerance law for special populations in Israel, which led to a broader detection of traffic violations in that population.

TUESDAY, September 1st

TuOP 7 IS THC-COOH-GLUCURONIDE A USEFUL MARKER FOR TETRAHYDROCANNABINOL (THC) IN DUID CASES?RETROSPECTIVE DATA ANALYSIS ON UPLC-HR-TOFMS DATA FILES FROM 2 YEARS OF DUID CASES.

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The physical and chemical nature of THC makes it difficult to include in traditional screening procedures along with other common legal and illegal drugs. Development of multi-component toxicological screening procedures that include THC is therefore a challenge but also highly desirable in high throughput laboratories.
The aim of the present study was to evaluate the detection of THC indirectly by detecting the presence of THC-COOH-glucuronide in whole blood from individuals suspected of driving under the influence of drugs (DUID). We will compare existing data from THC screening and quantification LC-MS/MS analysis with 2 years of UPLC-HR-TOFMS data by retrospective screening for THC-COOH-glucuronide.
Extraction was performed by simple protein precipitation of 300 µL whole blood with a mixture of acetonitrile and methanol containing deuterated internal standards. Ten microliters of the reconstituted extract were injected and separated within a 13.5 minute UPLC gradient run. HR-TOFMS instrumentation operated in positive electrospray ionization mode was used to generate the ions in the m/z range 50-1000. Fragment ions were generated by broad band Collision Induced Dissociation (bbCID). Identification was based on retention time, accurate mass, fragment ion(s) and isotopic pattern. The method is used routinely in our lab for a targeted screening in whole blood for approximately 400 compounds. The retrospective screening for THC-COOH-glucuronide was performed using TASQ data analysis software from Bruker. The Screening for THC-COOH-glucuronide included 1608 DUID whole blood samples from 2013 and 2014 already quantified on an existing validated LC-MS/MS method using Multiple Reaction Monitoring (MRM). The Danish per se limit for THC in DUID cases is 1.0 µg/kg blood and to compensate for variations between laboratories a 50 % uncertainty is added. That means that a driver is prosecuted for driving under the influence if the THC concentration in blood exceeds 1.5 µg/kg.
All cases with a concentration above 1.5 µg/kg THC (measured by LC-MS/MS) were screened positive by HR-TOFMS for THC-COOH-glucuronide (n=914). Of the cases with concentrations between 1.0 and 1.5 µg/kg (n=109) 95% were screened positive for THC-COOH-glucuronide. Opposite the cases with concentrations below 1.0 µg/kg (n=585) demonstrated a negative detection rate of 96%.
The HR-TOFMS screening method used routinely for screening a wide range of toxicologically relevant compounds can be used for screening for THC-COOH-glucuronide as an indirect marker of THC. All cases found with a THC level above the per se limit of 1.5 µg/kg were screened positive for THC-COOH-glucuronide on the HR-TOFMS method. This concludes that no cases above the DUID limit would have been omitted if the HR-TOFMS screening procedure had been used for deciding which samples were to be quantified on a more specific method.

TuOP 8 DRIVING UNDER THE INFLUENCE OF ALCOHOL AND ILLICIT DRUGS. A FIVE-YEAR STUDY ON 6500 DRIVERS INVOLVED IN ROAD ACCIDENTS IN NORTH-EAST ITALY

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Driving under the influence (DUI) of alcohol and illicit drugs represents a well-known risk factor in all kind of transportation accidents. Deterring from DUI of alcohol and drugs is therefore of utmost importance to improve road safety.
This study describes the law enforcement protocol devised to combat the DUI phenomenon in three provinces of the Veneto region, north-east Italy, and assesses the prevalence of alcohol and illicit drugs, based on blood samples, in drivers involved in road accidents during a five-year period.
Three blood and urine samples were collected, on police request, from each driver involved in road accident, using evacuated gray stopper tubes, in 20 hospital emergency departments, and sent to the Authors' laboratory with a proper chain of custody. Samples were analyzed for alcohol and illicit drugs (opiates, cocaine, cannabinoids, amphetamines, methadone) by means of immunoassay and MS techniques (HS-GC-MS, GC-MS, UHPLC/HR-MS), storing counter-analysis aliquots.
Law enforcement ascertainments were carried out in the provinces of Venezia, Treviso, and Rovigo (total population about 2 million people and geographical area of 6700 km2) of the Veneto region, according to the protocol implemented by the Authors, in agreement with regional courts, prefectures, and hospitals. 6500 blood and 5900 urine samples were collected and analyzed from drivers, injured or not in road accidents, during the period april 2010 – march 2015. Police requests for alcohol and drugs testing were 82.5%, for alcohol only 13.1%, for illicit drugs only 4.4%. Overall, 22.9% of blood samples were positive for alcohol (cut-off 0.1 g/L), 10.0% for drugs, and 4.0% for alcohol plus drugs. Blood alcohol concentrations (BAC) higher than 1.5 g/L were observed in the majority of alcohol-positive cases (44.7%). BAC levels in the ranges 0.1–0.5 g/L, 0.5–0.8 g/L and 0.8–1.5 g/L were 16.2%, 10.6% and 28.4% of the total cases, respectively. A decreasing trend of the percentage of drivers positive for alcohol was observed from 2011 (25.9%) to 2015 (21.1%). Tetrahydrocannabinol represents the most frequent found illicit drug (41.3%) in drug-positive cases, followed by cocaine (33.5%), opiates and methadone (9.5% and 10.0%, respectively), and different amphetamines (4.9%). Poly-drug use (mainly tetrahydrocannabinol and cocaine), and association of alcohol with drugs like tetrahydrocannabinol or cocaine or both, represented relatively common findings.
Results indicate that DUI of alcohol and illicit drugs still represents an important risk factor for road accidents in north-east Italy. However, the slight decrease of alcohol prevalence in drivers involved in road accidents over time could be due to the implementation of the described law enforcement protocol, as well as the growing awareness, especially among young people, of the danger of DUI of psychoactive substances.

11.00-1.00 -pm SESSION 2 - Antidoping

TuOP 9 LIPOSOMES AS DRUG DELIVERY SYSTEMS IN SPORT DOPING: RECENT ADVANCES IN THE STUDY OF LIPOSOME VEHICULATED HEMOGLOBINS

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The last years have signed the traces of a scientific debate considering the actual efficacy of the anti-doping testing programs, based on the view that the overall number of adverse analytical findings reported worldwide by the anti-doping laboratories accredited by the World Anti-Doping Agency appears to significantly underestimate the actual number of doped athletes. Many factors may be responsible of this gap, and one of them considers the potential effect of "doping masking agents", a multifaceted group of substances and methods that may be used with the aim to reduce the efficacy of the doping control test.
Drug delivery systems (DDSs) are artificial or entirely synthetic macromolecular structures designed to act as drug carriers, with the consequence that the pharmacokinetics of the carried drug becomes secondary to that of the carrier itself. It is not unlikely that DDSs have been or are being considered also for their potential effects as masking agents in sport doping. The present study is aimed to assess whether liposomes,a peculiar class of DDSs, may be effective as masking agents in sport doping, making more problematic the detection of the carried drug. At the same time, methods to detect the intake of liposomes were also studied.
Experiments were performed on banned substances belonging to the class of hemoglobin based artificial oxygen carriers (HBOCs). Determination of hemoglobin (free and cellular) was performed by automated hematological hemanalyzers (ADVIA), while detection and quantitative determination of liposomial-encapsulated hemoglobins (LEH) was performed in reference solution and in real samples by specifically developed techniques, based on flow cytofluorimetry with dual color fluorescence detection. More in details, hemoglobin detection was based on fluorescein isothiocyanate (FITC)-conjugated anti-hemoglobin antibodies, whereas biotin-conjugated anti-PEG antibodies coupled to Streptavidin-phycoerythrin (SA-PE) secondary antibodies were used to detect pegylated liposomes .
Our results indicate that liposomes are able to cause a reduction in the measured concentration of free hemoglobin, and that LEH is not detected by the current reference techniques used for the detection of HBOCs. This effect can both mask the intake of HBOCs and affect the significance of hemoglobin measurement as it is presently performed longitudinally in the framework of the so called hematological module of the "Athlete Biological Passport" (ABP). At the same time, methods to detect LEH by dual color flow cytofluorimetric techniques were allowing the identification of both pegylated liposomes and liposome-encapsulated hemoglobins (LEH), allowing sufficient sensitivity (1% of total hemoglobin) to detect the illicit intake of LEH.
We believe drug delivery systems should be considered in doping control analysis, methods to detect their presence in biological fluids should be available especially in the case of confirmation analysis of threshold substances.

TuOP 10 "DILUTE-AND-INJECT" MULTI-TARGET SCREENING ASSAY FOR (NOVEL) HIGHLY POLAR DOPING AGENTS USING HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY HIGH RESOLUTION/HIGH ACCURACY MASS SPECTROMETRY FOR SPORTS DRUG TESTING.

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In the field of LC-MS, reversed phase liquid chromatography (RPLC) is the predominant method of choice for the separation of prohibited substances from various classes in sports drug testing. However, highly polar and permanently charged analytes still represent a challenging task in liquid chromatography due to their difficult chromatographic behavior using RP materials. A very promising approach for the separation of highly polar compounds is hydrophilic interaction liquid chromatography (HILIC). However, HILIC is up to now not very common in doping analysis.
The aim of the study was to develop a multi-target LC-MS approach for the screening of various polar stimulants, stimulant sulfo-conjugates, glycerol, AICAR, ethyl glucuronide (ETG) and morphine-3-glucuronide (M3G) without a complex and time-consuming sample preparation. Furthermore, two novel highly polar compounds, bearing great potential for misuse in professional sports (myo-inositol trispyrophosphate (ITPP) and mildronate) were implemented in the assay to estimate their prevalence and extent of misuse internationally.
For sample preparation, urine samples were fortified with labeled internal standards and subsequently diluted using ammonium acetate solution (100mM) and acetonitrile (3:1:7, v:v:v). An effective online sample clean-up and preconcentration was accomplished using a dual pump setup in combination with a HILIC trapping column and a HILIC analytical column. After 1 min of isocratic elution on the trapping column, the system was backflushed using gradient elution from the trap to the analytical column. A Hybrid Quadrupole-Orbitrap® mass spectrometer, interfaced by an electrospray ionization ion source, operating in both positive and negative mode enabled the detection of highly polar analytes in full-scan, all ion fragmentation (AIF) and targeted higher energy collision dissociation (t-HCD) acquisition modes.
The methodology was fully validated for qualitative and quantitative (AICAR, glycerol) purposes considering the parameters specificity, robustness (rRT < 2.0%), linearity (R^2> 0.99), intra- and inter-day precision at low, medium and high concentration levels (CV < 20%), limit of detection (stimulants/stimulant sulfo-conjugates < 10ng/mL;

norfenefrine, octopamine < 30ng/mL; AICAR, mildronate < 10ng/mL; glycerol < 100µg/mL; ETG < 100ng/mL; M3G < 15ng/mL; ITPP < 20 ng/mL), accuracy (AICAR: 103.8-105.5%; glycerol: 85.1-98.3%) and ion suppression/enhancement effects. As a proof of concept, post-administration urine samples demonstrated the fit-for-purpose of the analytical assay. Furthermore, the present study indicates the wide prevalence of mildronate in international elite sports and demonstrates an alarming extent of administered dosages, providing urinary concentrations of more than 1 mg/mL.
To our knowledge, this is the first time that ITPP and mildronate were implemented in a multi-target screening approach. The combination of full-scan, AIF and t-HCD acquisition modes of the applied Hybrid Quadrupole-Orbitrap® mass spectrometer with between-scan polarity switching provides high sensitivity as well as the opportunity of retrospective data analysis. According to the flexibility of the present approach, the implementation of further highly polar compounds with difficult chromatographic behavior on common RP phases seems promising. As the state-of-the-art chromatographic technique for highly polar compounds, HILIC should receive more attention in sports drug testing.

TuOP 11 APTAMER BASED DETECTION OF PEPTIDE AND PROTEIN DOPING THREATS IN SPORT USING GONADOTROPIN-RELEASING HORMONE AND ERYTHROPOIETIN AS MODEL COMPOUNDS

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There is an increasing prevalence of naturally occurring biological molecules, such as peptides and proteins, being employed as doping agents in sport. Traditionally, antibodies have been used to detect these compounds as they enable purification and concentration of the analyte of interest while providing the required selectivity. The rapid expansion of peptide-based therapeutics necessitates a similarly rapid development of suitable affinity reagents. Aptamers, single stranded DNA oligonucleotides, may offer an alternative to antibodies in the enrichment of peptide and protein targets from biological matrices. Whilst the typical production time for antibodies ranges from 3 to 8 months, aptamers can be produced within 6-8 weeks and offer the significant advantage of rapid chemical synthesis once the nucleic acid sequence is known, negligible batch-to-batch performance variability, increased stability and shelf-life, and the ability to functionalise these affinity reagents more easily than antibodies.
The aim of the present study was to assess the quantitative detection of gonadotropin-releasing hormone (GnRH) in equine urine and the qualitative detection of erythropoietin (EPO) analogues in equine plasma using aptamer based enrichment strategies coupled with liquid chromatography tandem mass spectrometry (LCMSMS). The effectiveness of this strategy was compared to traditional immunoaffinity-enrichment strategies.
Biotin functionalized aptamers, with specific affinities towards GnRH and EPO, were bound to streptavidin coated paramagnetic beads and incubated with spiked equine urine and plasma samples. The beads were separated from the sample matrix using a magnet followed by elution of the target peptide and proteins and subsequent analysis by nano-LCMSMS.
Comparison of the validated aptamer enrichment method's performance to that of a published antibody enrichment method, for the detection and quantitation of GnRH in equine urine, shows good correlation between the two methods. Both methods achieved comparable limits of detection (1 pg/mL) and quantitation (2.5 pg/mL), together with intra- and inter-assay precision of better than 10% at 5 pg/mL and 20 pg/mL. In contrast, the two methods diverged when considering the dynamic range and the influence of the matrix on recovery and electrospray signal. The aptamer method outperformed the antibody method with regards to dynamic range (2.5-100 pg/mL and 2.5-40 pg/mL, respectively). However, the aptamer method showed significant matrix effects (170-174%) and low recovery (28-29%) where as the antibody method showed negligible matrix effects (90-103%) and greater recovery (45-48%).
The use of aptamers for the enrichment of the peptide GnRH from biological matrices suggests that aptamers may be a viable alternative to antibodies. The applicability of this approach to the qualitative detection of EPO and synthetic analogues will be discussed.

TuOP 12 ISOLATION, ENRICHMENT AND DETECTION OF ERYTHROPOIETIN MIMETIC AGENTS FROM HUMAN URINE BY MEANS OF EPO-RECEPTOR-PURIFICATION AND LC-HR-MS/MS DETERMINATION

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Erythropoietin and its recombinant and biopharmaceutically advanced forms unify the ability to bind and dimerize the EPO-receptor (EPOR) monomers with a subsequent activation of the JAK-STAT pathway to stimulate erythropoiesis. Thereby, the predictable side effect to increase endurance performance as a consequence of an increased oxygen supply makes these kinds of glycoproteins attractive for illicit doping purposes. Furthermore, new forms of EPOR binding peptides (erythropoietin mimetic peptides; EMP) may refuse the high avidity of anti-EPO monoclonal antibodies impeding the analysis of traces of new erythropoietin mimetic agents (EMA).
Former investigations have shown the benefits to isolate and enrich cytokines and other members of proteohormones (i.e. interleukins) from aqueous solutions by means of receptor-ligand interactions. The methods' proof-of-concept was to enlarge the opportunities of EMA analysis from human matrices in the context of comprehensive receptor affinity purification compensating the restricted specificity of monoclonal antibodies for erythropoietin mimetic agents and future upcoming EPOR agonists. Proceedings in the EMP-drug development and the emergence

of modern EMP carrier molecules necessitated to introduce a generic detection method for the crucial part of those designated bioactive oligopeptidic structures.
Aliquots of 15 mL of EMA spiked human urine were purified by EPOR-Fc magnetic beads and analyzed with commonly employed gel-electrophoretic separation processes (SAR-PAGE) and specific bottom-up analytics by means of ultra performance liquid chromatography / high resolution-high accuracy tandem mass spectrometry. Thereby, epoetin zeta, darbepoetin alfa, C.E.R.A. and peginesatide were utilized as model substances in representative urinary concentrations. Additionally, a cysteine specific bottom-up procedure via 2-nitro-5-thiocyanatobenzoic acid (NTCB) for all EMP structures of interest, in particular peginesatide, was introduced.
For epoetin zeta, darbepoetin alfa and C.E.R.A. the method was characterized for essential parameters (limit of detection (LOD), specificity and adsorption/matrix effects) to emphasize the tests' reliability. The characterization for peginesatide was accomplished and characterized in concordance to the ICH guidelines, consisting of the parameters specificity, linearity, precision, recovery, stability and ion suppression. In summary, the collected data showed an unambiguous detection for all examined recombinant EPO forms and peginesatide after EPOR pretreatment, respectively. Epoetin zeta, darbepoetin alfa and C.E.R.A. revealed limit of detections of 20, 30 and 80 pg/mL. The respective LOD for peginesatide in urine was 250 pg/mL. In all urine specimens the matrix effects and interferences were negligible.
The linkage of a human EPOR-Fc hybrid protein to magnetic beads provides an easy to handle new form of screening method to obtain drug candidates which are capable to bind and dimerize the EPOR monomers. Consequently, the here presented study contributes to enlarge the opportunities of protein isolation and purification from human matrices. Already existing detection methods to distinguish between the recombinant erythropoietins were conductively integrated showing the adaptability of the assay.

TuOP 13 FULL-LENGTH MGF AND XENON - NEW ADDITIONS TO ANTI-DOPING TEST PROGRAMS

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In 2014, doping control authorities and laboratories were confronted with new analytical challenges of particularly diverse nature. First, an unknown proteinaceous substance referred to as „full-length mechano growth factor" (full length MGF) was offered to ‚interested' elite athletes, supplied with the claim that a) it is undetectable and b) it is substantially more potent than conventional MGF. While conventional MGF products have been covered by routine doping controls before, a full-length MGF was yet to characterize and implement into sports drug testing. Shortly after the discovery of this product, journalists from The Economist revealed the use of xenon as performance-supporting agent amongst Russian athletes, being part of tournament preparations since the Athens Olympic Games in 2004. Until then, xenon was not covered by the World Anti-Doping Agency's (WADA's) Prohibited List and urged the Agency to modify its regulations instantaneously. For both issues, accredited doping control laboratories had to find solutions from an analytical angle, preferably exploiting existing sports drug testing matrices.
The aims of the study were establishing analytical means to test for the newly identified full length MGF and the gaseous analyte xenon.
HRMS hyphenated to liquid chromatography and gas chromatography was used to identify, characterize, and test for the higher molecular mass analyte full length MGF and the low molecular mass compound xenon, respectively. The peptidic analyte required a dedicated sample preparation protocol adapted from existing methodologies to enable adequate purity for subsequent nanoLC-HRMS analysis. In the absence of authentic elimination study samples, spiked specimens were prepared and extracted by ultrafiltration and immunoaffinity purification. For xenon, GC-HRMS and -MS/MS was used after either blood, plasma, or urine was enriched with deuterated hexanone as internal standard and subjected to headspace sample injection. Here, authentic patient samples collected after xenon-assisted anesthesia were used for proof-of-concept specimens. Both test methods (i.e. for full length MGF and xenon) were validated and their fitness-for-purpose assessed.
Full length MGF was identified as a modified version of IGF-I isoform 4, prohibited by WADA's regulation under the umbrella of ‚related substances'. Due to its non-natural modification and formerly unknown structure, its detection in doping control samples would have been unlikely in cases of illicit use by athletes. Yet, the compound's metabolism and elimination is not clarified and further studies are warranted. However, using adapted approaches, a detection limit of 0.25 ng/mL was accomplished.
Xenon was successfully analyzed from spiked matrices as well as identified from authentic blood and urine samples obtained from patients undergoing xenon-assisted anesthesia. The narcotic agent was traceable for up to 40 h using the established approaches, which were fully validated and LODs were 0.5 nmol/mL for both blood and urine. New analytes relevant for doping controls had to be characterized and implemented into routine sports drug testing programs necessitating adaptations to sample preparation procedures as well as mass spectrometric assays. Appropriate detection limits were accomplished suggesting sufficient windows of opportunity for doping control laboratories. Since the implementation of the analytes into analytical methods, no adverse analytical finding has yet been reported.

TUESDAY, September 1st

TuOP 14 ADIPOR AGONISTS AND THEIR IMPLEMENTATION IN SPORTS DRUG TESTING

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AdipoR agonists are small, orally active molecules capable of mimicking the protein adiponectin, which represents an adipokine with antidiabetic and antiatherogenic effects. Two adiponectin receptors were reported in the literature referred to as adipoR1 and adipoR2. Activation of these receptors stimulates mitochondrial biogenesis and results in an improved oxidative metabolism (via adipoR1) and increased insulin sensitivity (via adipoR2). Hence, adipoR agonists are potentially performance enhancing substances and targets of proactive and preventive anti-doping measures.
The aim was to synthesize and characterize the two known adipoR agonists termed AdipoRon and 112254 as well as two isotopically labeled analogs, respectively. With these compounds, a qualitative liquid chromatography-high resolution mass spectrometry (LC-HRMS) method in human plasma should be established and fully validated for doping control purposes. Also, metabolites of the adipoR agonists were determined by in vitro-studies.
Structures of both compounds were confirmed by NMR and high-resolution/high-accuracy mass spectrometry (HRMS). Product ions after collision-induced dissociation (CID) were analyzed. Density functional theory (DFT) calculations were made to determine the site with the highest proton affinity. For the sample preparation for the validation both compounds and the labeled analogs acting as internal standards (ISTD) were spiked in human plasma. The proteins were precipitated before the sample was injected into a liquid chromatography-tandem mass spectrometry-system (LC/MS/MS). The substances were validated regarding specificity, LLOD, identification capability, robustness, carryover, recovery, linearity, interday and intraday precision and matrix interferences. To determine metabolites both adipoR agonists both were incubated with human liver microsomes and the S9-fraction to identify potential phase I and II metabolites.
All product ions found after CID were confirmed by accurate mass, H/D-exchange experiments, DFT-calculations and by CID-experiments of the synthesized labeled compounds. For Validation out of human plasma, specificity was determined by analysis of 10 plasma samples, five male and five female samples. LLODs were determined at 1 ng/mL for both substances and the recovery was 89% for AdipoRon and 86% for 112254. Moderate ion suppression was found for AdipoRon whereas for 112252 nearly no ion suppression was observed. Interday and intraday precision were determined for both compounds at three concentration levels with the standard deviation ranging overall from 3-18%. Additionally, the qualitative parameters identification capability, robustness and carryover were determined.
The in vitro studies resulted in the exclusive formation of phase I metabolites. For both substances mainly the monohydroxylated metabolite, but also dihydroxylated, reduced or demethylated metabolites were found.
AdipoR agonists were prepared and implemented in doping control procedures as these therapeutics might possess potential for misuse due to their capability to increase the mitochondrial content in muscle cells. Also, studies to identify potential phase I and II metabolites were conducted, providing viable target analytes for doping controls.

TuOP 15 MYOSTATIN INHIBITORS IN SPORTS DRUG TESTING: DETECTION OF MYOSTATIN-NEUTRALIZING ANTIBODIES IN PLASMA SAMPLES BY USING IMMUNOAFFINITY PURIFICATION AND WESTERN BLOTTING

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Myostatin (growth/differentiation factor-8, GDF-8) is a highly conserved member of the transforming growth factor-β (TGF-β) superfamily which functions as a negative regulator of skeletal muscle mass in both developing embryonic and adult tissue. Specific inhibitors binding either to myostatin itself or the activin type II receptors may provide important therapeutic approaches for the treatment of muscular diseases (e.g. muscular dystrophies) and have also been discussed as performance-enhancing agents in sports.
The aim of this study was to develop a detection assay for myostatin-neutralizing antibodies, which can serve as proof-of-concept for the detection of myostatin inhibitors as well as therapeutic antibodies in doping control samples.
To simultaneously detect chimeric, humanized and human anti-myostatin antibodies irrespective of their amino acid sequence, an immunological assay specific for antibodies which are directed against myostatin and have a human Fc domain was established. Antibodies were isolated from plasma or serum by immunoaffinity purification and then subjected to western blot analysis. By using myostatin as bait protein, myostatin-neutralizing antibodies present in the purified antibody-mixture were captured and subsequently detected by an enzyme-linked secondary antibody specific for the Fc fragment of human IgG. Following method development, the assay was validated according to parameters used for mass-spectrometry based methods employing the recombinant human anti-myostatin antibody MYO-029 as model compound.
The assay was found to be highly specific, robust, and linear in the concentration range of 0.05 to 1 µg/mL. The precision was successfully specified at three different concentration levels and the recovery of the immunoaffinity purification was 58 percent.
Within this study, an immunological detection assay for myostatin-neutralizing antibodies in doping control plasma or serum samples was developed and successfully validated. In theory, the assay is capable to simultaneously detect chimeric, humanized and human myostatin-binding antibodies irrespective of their amino acid sequence, and further therapeutic antibodies can be easily implemented by using other bait-proteins. The assay serves as proof-of-concept for the detection of myostatin inhibitors as well as therapeutic antibodies.

TuOP 16 INNOVATIVE CHEMOMETRIC APPROACHES AS POWERFUL TOOLS IN THE ATHLETE BIOLOGICAL PASSPORT

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Endogenous anabolic androgenic steroids (EAAS) have large diffusion as doping agents since the distinction between their endogenous (physiologic) production and exogenous administration is still difficult to ascertain. Therefore, EAAS keep being the most abundant misused substances in elite sports. The small changes of EAAS concentrations, induced from the intake of low but frequent doses of various EAAS at almost physiological levels, are masked by the wide inter- and intra-individual variability of urinary EAAS values. In order to identify EAAS misuse by athletes, WADA introduced into the Athlete Biological Passport (ABP) an adaptive statistical model based on Bayesian inference, aiming to detect abnormal values of selected EAAS such as: testosterone (T), epitestosterone (E), androsterone (A), etiocholanolone (Etio), 5α-androstane-3α,17β-diol (5α-diol), 5β-androstane-3α,17β-diol (5β-diol), and further EAAS ratios such as: T/E, A/T, A/Etio, 5α-diol/5β-diol and 5α-diol/E.
Our aims were i) to develop a GC-MS method addressed to quantify a wide range of endogenous steroids in urine, and ii) to develop and compare various multivariate statistical approaches based on effective combination of EAAS values and their ratios, in order to enhance the detection of alleged EAAS misuse by means of the Athlete Biological Passport (ABP).
Urine samples were taken from 108 subjects, including 12 patients under treatment with EAAS or finasteride. A fully-validated GC-MS method was developed in order to detect all EAAS recommended by WADA plus further endogenous steroids that are reported in literature as potential EAAS misuse markers or microbiological degradation markers. Collected data plus different ratios of the analytes were evaluated by means of three multivariate data analysis techniques, namely Principal Component Analysys (PCA), Unequal Dispersed Classes (UNEQ) and Partial Least Squares Discriminant Analysis (PLS-DA).
The 12 positive urine samples taken from subjects assuming EAAS or finasteride were all classified as negative if the standard screening criteria proposed by WADA were used. On the other hand, the application of the multivariate statistical techniques on EAAS profiles allowed us to correctly detect the drug intake and exclude these samples from the normal "negative" population. In particular, PLS-DA proved to be very efficient by showing both sensitivity and specificity equal to 100%.
According to our preliminary results, multivariate statistic modeling can be exploited on screening test data from anti-doping analyses for the assessment of EAAS misuse by athletes. The application of statistical techniques such as PLS-DA can productively be introduced into the Athlete Biological Passport in order to assist the correct classification of unknown samples, according to whether the fitted response is far or closer to an estimated multivariate threshold.

TuOP 17 DRUG-DRUG INTERACTIONS AND MASKING EFFECTS IN SPORT DOPING: THE CONTRIBUTION FROM IN VITRO STUDIES COUPLED TO CHROMATOGRAPHY-MASS SPECTROMETRY

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One of the most challenging tasks in anti¬doping science is the identification of potentially effective new masking agents and masking strategies, whose goal is to make more problematic the detection of doping agents and methods from the analysis of biological fluids.
This contribution considers the potential effects of drug-drug interactions on the detection of markers of administration of banned drugs in sport doping. In details, this study is aimed to verify whether the co¬administration of banned and non banned drugs can make more problematic the interpretation of analytical data.
Incubations were performed using human liver microsomes (HLM) and single cDNA expressed CYP450 and UGT enzyme isoforms to simulate in vitro the phase I and phase II metabolic reactions of anabolic androgenic steroids. Inhibition studies were carried out in the presence and in the absence of twelve non banned drugs (antifungal agents, benzodiazepines and non-steroidal anti-inflammatory drugs) commonly administered by athletes. Preliminary results were also obtained following in vivo studies and administration trials. Analytical measurements were carried out by both GC¬MS(/MS) and LC¬MS(/MS) based analytical methods.
Our data indicate that the measuring concentration of the two main conjugated metabolites of 19-nortestosterone (nandrolone) that are 19¬norandrosterone glucuronide and 19¬noretiocholanolone glucuronide are significantly reduced following incubation studies performed in the presence of non banned drugs. Furthermore, the presence of anti¬fungal agents also alters the measured concentration of the endogenous androgenic steroids measured in the framework of the urinary steroid profile (which, in turn, constitutes the basis for the steroidal module of the athlete biological passport).
The experimental evidence obtained in this study suggests that the selection of the most suitable target analyte for the detection of the illicit use of doping substances should be performed taking into account also the potential effects of drug-drug interactions. In the case of pseudo¬endogenous substances, careful monitoring of any unusual result should always be performed, considering the potential effects of non banned drugs.

TUESDAY, September 1st

2.30-4.00 pm SESSION 3 – Toxicokinetics III

TuOP 18 EVALUATION OF THE BINDING AFFINITIES OF 54 NEWLY-EMERGED SYNTHETIC CANNABINOIDS AT THE CANNABINOID CB1 AND CB2 RECEPTORS

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To avoid a cat-and-mouse game between the authorities and illicit-drug manufacturers, a comprehensive system (generic scheduling) for designating the naphthoylindole-type synthetic cannabinoids was added to the "Designated Substances" law in Japan in 2013. After the enforcement of the generic scheduling, these substances were rarely detected and were completely replaced by other types. In particular, the synthetic cannabinoids, whose structures are made up of carboxamide or carboxylate ester, entered the drug market. Most of them had not yet had their pharmacological activities reported.
The aim of the present study was the measurement of the binding affinities of 54 newly-emerged synthetic cannabinoids at the cannabinoid CB1 and CB2 receptors and the evaluation of the relationships between their chemical structures and binding affinities.
The 54 synthetic cannabinoids, which newly emerged in these three years and had not yet had their pharmacological activities reported (except a typical naphthoylindole-type synthetic cannabinoid; JWH-018), were investigated. The IC_{50} values of these substances were measured based on the competitive interaction between a labeled ligand ([3H] CP-55,940) and an analyte using the membrane target system prepared from CHO-K1 cells that express the recombinant human cannabinoid CB1 or CB2 receptors (PerkinElmer).
Most of the test substances had almost the same or higher CB1 and CB2 receptor binding affinities as compared to JWH-018. Among the 54 substances investigated in this study, the structures which consisted of indole/indazole–carboxamide–3-methylbutanamide/3,3-dimethylbutanamide (e.g. 5F-ADB-PINACA and AB-CHMINACA), indole/indazole–carboxamide–methyl 3-methylbutanoate/methyl 3,3-dimethylbutanoate (e.g. MDMB-FUBINACA, MDMB-CHMINACA, MDMB-CHMICA, 5F-ADB and 5F-AMB) and indole/indazol–carboxylate ester–quinolinyl (e.g. 5F-PB-22 and FUB-PB-22) revealed the highest affinities at the CB1 receptor. In particular, the IC_{50} values of 5F-ADB-PINACA, MDMB-FUBINACA and 5F-PB-22 were more than 175 times greater than that of JWH-018. These substances had also high binding affinities at the CB2 receptor. The indazole analogs of an indole moiety tended to have higher affinities than indole-type substances, except the structures consisted of Indole/indazole–carboxamide–adamantyl (e.g. APICA/APINACA). Moreover, the N-fluoroalkyl analogs of an indole/indazole moiety had higher affinities than N-alkyl compounds. As a result of our previous survey based on the chemical analyses of herbal or powdery products obtained in Japan from 2013 to 2014, 5F-PB-22 and FUB-PB-22 were the most popular in 2013 and early 2014, respectively. 5F-AB-PINACA, AB-CHMINACA and 5F-AMB were also popular in mid-2014, and NM2201 was the most detected in the latter half of 2014, followed by 5F-ADB-PINACA. These results suggest that the distribution of synthetic cannabinoids may correspond to their possible pharmacological activities such as the CB1 and CB2 receptor binding affinities.
At the end of 2014, EMCDDA reported some fatalities in Sweden associated with MDMB-CHMICA, which was one of the substances with the highest binding affinities at the CB1 receptor in this study. Although the binding affinities at the CB1 and CB2 receptors do not directly reveal each pharmacological activity, the evaluation of these values will be useful for the prediction of their prevalence and potential health damage.

TuOP 19 CB1 AND CB2 RECEPTOR AFFINITIES OF SYNTHETIC CANNABINOIDS ON THE ILLICIT DRUG MARKET

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The internet drug market is flooded with steadily changing synthetic substances being consumed as alternative to natural cannabis products. The interpretation of analytical results of synthetic cannabinoids is difficult due to the missing of pharmacological and toxicological data. The potential of substances can be estimated by their binding affinity to the CB1 receptor compared to tetrahydrocannabinol (Ki (CB1) ~ 40.7 nM), however, for most of the newer substances affinities are not described.
Aim of this study was to determine receptor affinities and activation potential for CB1 and CB2 of synthetic cannabinoids on the illicit drug market.
For more than 25 substances (APICA; AB-005 Azepan isomer; A-796,260; A-834,735; ADBICA; 5F-AKB48 (5F-APINACA); STS-135; BB-22; FUB-PB-22; FDU-PB-22; MN-25; THJ-018; 5-F-THJ; XLR-11; XLR-(N-2-(fluoropentyl)-Isomer; XLR-12; UR-144; MAM-2201-5-F; MAM-2201-4-fluorpentyl-Analog; RCS-4; RCS-8; EAM-2201; THJ; CB-13; 5F-AKB; NNEI, 5-Fluoro-NNEI, 5-fluoro-AB-PINACA, AB-CHMINACA, THJ 2201) human CB1 and CB2 receptor Ki values were determined in heterologous radioligand binding assays vs. [3H]CP55,940 (0,1 nM), using membrane preparations stably expressing the receptor, for calculation the Cheng-Prusoff equation and a KD value of 2.4 nM ([3H]CP55,940 at hCB1) and 0.7 nM ([3H]CP55,940 at hCB2) were used. As a source for human CB1 and CB2 receptors membrane preparations of Chinese hamster ovary (CHO) cells were used. Additionally, inhibition of adenylate cyclase activity was determined in CHO cells using a competition binding assay for cAMP.
Most of the substances are full agonists at cannabinoid receptors. Highest affinities at CB1 showed BB-22 (Ki (CB1) = 0.217 ± 0.56 nM), EAM-2201 (Ki (CB1) = 0.380 ± 0.111 nM) and analogues, PB-22 analogues like FUB-PB-22 (Ki

(CB1) = 0.386 ± 0.117 nM) and ADBICA (Ki (CB1) = 1.87 ± 0.20 nM), 5F-AKB48 (Ki (CB1) = 2.07 ± 0.71 nM) and APICA (Ki (CB1) = 6.52 ± 3.73 nM). THJ and analogues, XLR substances, RCS4 and RCS8 showed moderate affinity to hCB1 but higher selectivity to hCB2.
Presented receptor affinities should be integrated in interpretation of analytical results regarding synthetic cannabinoids. Further substances will be tested until the start of this meeting.

TuOP 20 CYP2D6 GENOTYPE-DEPENDING CHIRAL PHARMACOKINETICS AND PHASE I AND II METABOLISM OF MDMA AFTER CO-ADMINISTRATION OF BUPROPION IN A CONTROLLED STUDY IN HUMANS

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3,4-methylenedioxymethamphetamine (MDMA) metabolism is known to be stereoselective, mostly with preferences for the S-stereoisomers. Its major metabolic step involves CYP2D6-catalyzed demethylenation to 3,4-dihydroxymethamphetamine (DHMA), which can subsequently be methylated and/or conjugated. Altered CYP2D6 geno- and/or phenotype have been associated with higher toxicity of MDMA.
Plasma pharmacokinetics should be compared for all relevant MDMA metabolites after controlled MDMA administration to extensive (EM) and intermediate metabolizers (IM) as well as after additional administration of bupropion, a known CYP2D6 inhibitor.
Blood plasma samples were collected up to 24 h after administration from 16 healthy participants (13 EM, 3 IM) of a double-blind, placebo-controlled, four-period cross-over study receiving one week placebo or bupropion pretreatment (150 mg) followed by a single placebo or MDMA 125 mg dose. Analysis was performed using two different LC-MS/MS methods (ABSciex 5500 Qtrap; Dionex UltiMate 3000; Phenomenex Kinetex C18 column/Chiralpak AGP column) after protein precipitation (Steuer et al, DTA, 2014) for chiral analysis of MDMA and bupropion, respectively. The study was conducted in accordance with the Declaration of Helsinki and approved by the local ethics committee (NCT01771874). Noncompartmental analysis was used to determine PK parameters using PK solutions 2.0 software. Pharmacokinetic parameters were compared (Kruskal-Wallis test, $p<0.05$).
Statistically significant differences were observed in Cmax and AUC0-24h for R,S- DHMA 3-sulfate, R,S-DHMA 4-sulfate, R,S-HMMA sulfate and R,S-HMMA glucuronide with mean decreased values to approximately 30% for the IM compared to EM and after co-administration of bupropion compared to placebo, respectively. Both stereoisomers were influenced to the same extent and R/S-ratios were not altered by different genotypes or by co-ingestion of bupropion. MDMA Cmax and AUC0-24h increased, but differences were not statistically significant. No relevant differences could be observed for MDA. Generally, pharmacokinetics in IMs was comparable to that obtained after bupropion pretreatment. Metabolite ratios of any MDMA metabolite to MDMA were lower with decreased CYP2D6 function. Variations over time were bigger than those caused by different CYP2D6 activity. No correlation could be observed between AUC0-24h of R- or S-bupropion, R,R-or S,S-HO-bupropion or dehydrobupropion and AUC0-24h ratios of any MDMA metabolite/MDMA.
As expected, changes in CYP2D6 lead to decreased formation of all MDMA metabolites resulting from the initial demethylenation of MDMA. Inhibition of CYP2D6 by bupropion resulted in comparable changes in pharmacokinetics as observed for IM vs. EM. Both stereoisomers were affected to the same extent, resulting in comparable R/S ratios independent of CYP2D6 activity.

TuOP 21 HEROIN KINETICS IN BLOOD AND VITREOUS HUMOR IN A LIVING PIG MODEL

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In forensic toxicology, blood concentrations of morphine and 6-monoacetylmorphine (6MAM) are often used to determine both the cause of death and the time elapsed between drug administration and death in heroin fatalities. Vitreous humor (VH) is a specimen that shows less variation in drug concentrations after death than blood, and thus a valuable specimen in interpreting postmortem drug levels. The ratio blood/VH of postmortem concentrations of heroin metabolites has been described in some studies, but little is known about the time course of the transfer into VH in the living organism. A better understanding of the pharmacokinetics of these substances might open new perspectives about the utility of VH as a complementary sample in forensic toxicology.
The aim of this study was to investigate the changes over time of the concentrations of morphine and 6MAM in VH after intra venous (i.v.) administration in the living organism.
Six pigs, weight 45±5 kg were given general non opioid anesthesia. Respiration and other vital functions were monitored throughout the study period. A micro dialysis probe was placed in the eye to sample dialysate from VH, and a catheter was implanted in the central vena cava for blood sampling. A bolus dose of 20 mg i.v. heroin was administered and multiple samples taken during a period of 360 minutes before euthanasia. The samples were analyzed by UPLC-MS/MS. Results are presented from fitting curves calculated by a population method using the program Kinetica 5.1.
After i.v. injection of 20 mg heroin, the blood Cmax of 6MAM was 2.01 µM, reached after 1 minute. The concentration then decreased to 0.017 µM after 120 minutes. In VH, 6MAM was first detected after 60 minutes, the Cmax of 0.024 µM was reached after 230 minutes. Thereafter the concentration slightly decreased to 0.020 µM after 360 minutes. The blood Cmax of morphine was 0.37 µM, reached after 5 minutes. The concentration then decreased to 0.017 µM after 360 minutes. In VH, morphine was first detected after 60 minutes and increased during the total experimental

period thereafter, i.e. until a Cmax of 0.022 µM was reached after 360 minutes. The blood/VH ratio for morphine according to the fitted curve was 160 after 1 hour, 4 after 2 hours and decreased to approximately 1 after 6 hours. In the living animal, morphine and 6MAM have a slow transport to VH and equilibrium with blood is not reached before 2-3 hours. Detection of 6MAM in VH does not necessarily reflect a very recent intake of heroin. Regarding the ratio between morphine concentrations in blood and VH, this is extremely high the first 2 hours, but in the 2-6 hours after injection, the ratio between blood and VH is close to 1. As post mortem changes are expected to be small in VH, these results could assist the interpretation of post mortem morphine blood results.

TuOP 22 SEROTONIN TOXICITY AND CYTOCHROME P450 GENETIC POLYMORPHISMS

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Serotonin toxicity commonly occurs in the context of serotonergic drug overdose or from interactions between multiple serotonergic agents. Fluoxetine is a serotonergic agent of the selective serotonin reuptake inhibitor (SSRI) class. Evidence from several in vitro and in vivo studies indicates the involvement of CYP2D6, CYP2C19 and CYP2C9 in the biotransformation of fluoxetine. Moreover, fluoxetine has demonstrated inhibitory potency toward CYP2C19, CYP2C9, and CYP3A4, consequently having the potential to alter metabolism and pharmacokinetics of co-administered drugs metabolised through the same pathway or its own metabolism.
To describe a case of serotonin toxicity from an inability to metabolise serotonergic agents due to poor activity of CYP2D6 and CYP2C19.
Informed consent was obtained from the patient for genetic testing of her CYP enzymes. DNA was extracted from blood using the manufacturer's protocol for the QIAGEN EZ1 BioRobot system. The variant alleles of CYP2D6*4 rs3892097, CYP2D6*5 (DEL), CYP2C19*2 rs4244285, CYP2C19*3 rs4986893, CYP2C9*2 rs1799853, CYP2C9*3 rs1057910, CYP1A2*1D rs35694136, CYP1A2*1F rs762551, CYP2D6*41 rs28371725, CYP3A4 rs2740574, CYP2D6*10(*4) rs1065852 that affect the function of cytochrome enzymes were genotyped. The genotyping method involves specific restriction enzyme digestion of amplified PCR products (PCR-RFLP). Fragment analysis is based on capillary electrophoresis Agilent Bioanalyser methodology.
We describe a 29-year-old woman with depression and anxiety who was commenced on regular fluoxetine 20mg/day for 7 weeks, followed by 40mg/day for 3 weeks. During the 10-week period she began to have increasing agitation and episodes of flushing, sweating and tremor. She presented to the emergency department and was initially diagnosed with a panic attack only to represent 7 hours later with ongoing diaphoresis, tachycardia (122 beats per minute), hypertension (147/102 mmHg), exaggerated resting tremor and inducible clonus. Her clinical presenation satisfied the Hunter criteria for serotonin toxicity. Following cessation of fluoxetine and treatment with diazepam, her symptoms improved and she was discharged the following day. Restriction Fragment Length Polymorphism assay (PCR-RFLP) revealed a presence of homozygote rs3892097 and rs1065852 polymorphisms in CYP2D6 and heterozygote 2C19 rs4244285/rs4986893 polymorphisms, which are associated with poor metaboliser phenotype and presence of one copy of CYP1A2 rs35694136 and rs762551.
This patient genotype is a very rare case of combined loss-of-function of CYP2D6 and CYP2C19 enzymes. In addition, heterozygote polymorphisms, which are associated with impaired activity of CYP1A2, possibly contribute to low activity of the cytochrome P450 drug metabolising pathway. The involved cytochrome P450 isoforms exhibit genetic polymorphisms that affect their catalytic activity.

TuOP 23 FIRST METABOLIC PROFILE OF PV8, A NOVEL SYNTHETIC CATHINONE, BY HIGH-RESOLUTION MASS SPECTROMETRY (HRMS)

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Novel psychoactive substances (NPS) are constantly emerging onto the illicit drug market making it difficult to identify new cathinones and their metabolites. Recently, PV8, a synthetic pyrrolidinophenone, was identified in illegal products purchased in Japan in 2013 (Uchiyama et al 2014) and in seized products in the Netherlands and Germany (www.designer-drugs.de). The only available reports of PV8's effects are from internet drug forums suggesting similar potency to α-PVT and α-PVP; as for many NPS, there are no controlled PV8 administration studies, and no other available pharmacodynamic and pharmacokinetic data. One important reason there are no data is because there are no PV8 metabolism studies to identify unique PV8 intake markers. If PV8 urinary markers were known, observed adverse effects could be tied to PV8, and the public could be educated on its dangers.
The objective was to determine PV8's metabolic stability with human liver microsome (HLM) incubation and its metabolism with human hepatocyte incubation and HRMS.
To determine half-life for selecting hepatocyte incubation, PV8 was incubated with pooled HLM for up to 1h. HLM samples were diluted 100-fold with mobile phase A (0.1% formic acid in water), and separated on an Accucore C18 column (100 x 2.1mm, 2.6µm) within 20min. For metabolite profiling, drug was incubated with pooled cryopreserved human hepatocytes for up to 2h. Hepatocyte samples were diluted 5-fold with mobile phase A, separated on a Synergi Hydro-RP column (150 x 2mm, 4µm) within 30min. All samples were analyzed with a Thermo Scientific Q-Exactive HRMS. Data were acquired full-scan, and with a data-dependent mass spectrometry method. Analysis of hepatocyte samples also utilized an inclusion list of predicted metabolite masses generated by the in silico MetaSite software (v. 4.2.2). Additionally, hepatocyte samples also were acquired with an all-ion-fragmentation mass spectrometry method to identify potential unexpected metabolites. Scans were thoroughly data mined with

different data processing algorithms and analyzed in WebMetaBase.
PV8 exhibited a relatively short 28 min half-life, with an intrinsic 24-µL/min*mg clearance. This compound is predicted to be an intermediate-clearance drug with an estimated human 23.0-mL/min/kg hepatic clearance. Metabolic pathways included: iminium formation, aliphatic hydroxylation, ketone formation, N-dealkylation, N-dealkylation and ketone reduction, and aliphatic hydroxylation and glucuronidation. The top 3 metabolic pathways with hepatocytes were di-hydroxylation > ketone reduction > hydroxylation. Several glucuronidated metabolites also were identified in the hepatocyte incubations. The top 3 metabolites generated in silico were hydroxylated metabolites (aliphatic and aromatic).
For the first time, PV8 metabolites were identified and structurally elucidated by HRMS. These PV8 metabolite spectra can be incorporated into liquid chromatography MS urine screening methods for identifying PV8 intake during forensic and clinical testing. These data provide useful urinary metabolite targets and also focus reference standard companies' synthetic efforts to provide commercially available standards needed for PV8 testing. Supported by the National Institutes of Health, Intramural Research Program, NIDA

TuOP 24 FLUBROMAZOLAM - CHARACTERIZATION AND BASIC PHARMACOKINETIC EVALUATION OF A HIGHLY POTENT DESIGNER BENZODIAZEPINE

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Benzodiazepines play an important role in forensic and clinical toxicology as they are widely used for treatment of psychiatric disorders and as drugs of abuse. In 2012, the first designer benzodiazepines were offered in Internet shops providing an attractive and low priced alternative to prescription-only benzodiazepines. Data regarding pharmacokinetic parameters, metabolism and detectability in biological fluids is not available or limited.
This study was designed to characterize the designer benzodiazepine flubromazolam and obtain information on its in vitro metabolism. Furthermore, a self-experiment was performed to gain preliminary data on pharmacokinetic properties and to identify the main metabolites in vivo. The obtained biological samples were also used to assess the time frame of detectability and the performance of benzodiazepine immunoassays.
Flubromazolam was obtained as research chemical. For identification and characterization NMR spectroscopy, GC-MS, LC-MS/MS and LC-Q-ToF-MS were applied. The main phase I metabolites were investigated in vitro by using a pooled human liver microsome (pHLM) assay. Enhanced product ion (EPI) scan experiments as well as Q-ToF analysis were conducted. To assess basic pharmacokinetic data and in vivo metabolism one of the authors ingested a capsule containing 0.5 mg of flubromazolam. Several blood and urine samples were obtained pre and post intake. Using alprazolam-D_5 and OH-alprazolam-D_5 as internal standards an MRM method was set up for quantitation. All obtained serum and urine samples were tested for benzodiazepines using two different immunoassays (CEDIA on AU400, KIMS on cobas 501) adapted to forensic cut-offs.
The chemical structure of flubromazolam was confirmed by NMR spectroscopy and purity was estimated > 94 % (GC-MS). In the Q-ToF data and EPI scans of the pHLM assay signals corresponding to the m/z of a mono- and dihydroxylation were observed. In vivo only a mono-OH metabolite was detected. The volunteer experienced strong sedative effects starting about three hours post intake, lasting for at least 10 h. Additionally, he developed partial amnesia lasting far over 24 h. The first peak concentration of flubromalozam was reached after 5 h (7.4 ng/ml); a second peaked occurred after 8 h (8.6 ng/ml, post-prandial). The estimated terminal elimination half-life is in the range of 10 to 20 h. In all serum samples taken post ingestion the performed immunoassay yielded negative results, whereas the urine samples from 3 h up to 2 and 5 days post ingestion, respectively, were reported as positive with the applied assays.
Flubromazolam proved to be highly potent, thus bearing a high risk for being used in drug facilitated crimes. This is aggravated by the easy availability via online shops and its water solubility. In analogy to other triazolo-benzodiazepines, hydroxylation is most likely to occur at the α- and the 4-position. Detectability of flubromazolam uptake in serum samples using immunoassays seems very unlikely. Yet, in urine samples positive findings might be misinterpreted as 'false positive' whenever the confirmation method used does not include the target analyte. Therefore, targeted confirmation methods have to be kept up-to-date at all times.

TUESDAY, September 1st

4.30-6.00 pm SESSION 4 – Toxicokinetics IV

TuOP 25 SEQUENCING CYP2D6 IN POST-MORTEM BLOOD SAMPLES WITH HIGH CONCENTRATION OF TRAMADOL FOR THE DETECTION OF *3, *4, *6, *8, *10 AND *12 ALLELES

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Tramadol is a centrally acting opioid analgesic used very often in hospital for pain control. Tramadol is bioactivated in the liver by CYP2D6 enzyme to O-desmethyl-tramadol that has approximately 200 times more affinity to opioid receptors and is responsible for the main analgesic effect. CYP2D6 gene is located on the chromosome 22q13.1 and is highly polymorphic. More than 20 allelic variants are related to enzyme inactivation. Approximately 5 to 10% of the European population is homozygous for a defective CYP2D6 gene and allele *4 is the most frequent null allele. Some authors have demonstrated that there is a correlation between genotype and phenotype for tramadol.
To aid interpretation of the forensic toxicology results in tramadol positive cases, we have sequenced CYP2D6 in post mortem blood samples to identify some polymorphisms that cause absence of enzyme activity, specially the CYP2D6*4 allele (more prevalent in Caucasians).
DNA from post-mortem blood samples was extracted by Chelex 100® method and quantitated with Applied Biosystems (AB) Quantifiler Trio Kit ® by Real-time PCR. Fragments amplification was done by PCR and verified by PHASTGEL® gradient 10-15 electrophoresis. Sequencing was performed with Big Dye v.3.1 cycle sequence (AB) and analyzed in a Genetic Analyser 3130 (AB). Allelic variants were found comparing the results with a reference sequence (CYP2D6*1, GenBank entry M33388.1).
Post-mortem blood samples were genotyped for CYP2D6 *3, *4, *6, *8, *10 and *12. The prevalence of observed nonfunctional CYP2D6 allele 4* was 27.5%, which is higher than the expected, based on large European population studies. Allele *10 frequency was 7.5% and CYP2D6 *3, *6, *8 and *12 alleles were absent.
We found homozygosity for CYP2D6*4 allele in 3 samples. The 3 individuals with no functional CYP2D6 genes were predicted as poor metabolizers. These results may be used to explain the higher concentration of tramadol found, as well as the lack of analgesic effect due to lower O-desmethyl-tramadol concentrations, and can be important to exclude intoxication as cause of death, mainly in cases with administration with controlled dosage.
This study proved that the sequencing methodology can be successfully applied to the detection of genetic polymorphisms at CYP2D6 in post-mortem blood samples. With sequencing methodology is possible to identify more allelic variants already known (*14, *15, *19, *20, *41, *44) or other located in the same fragments that could be identified in the future, without further costs. This methodology can also be applied to cases with other substances with the same metabolic way (CYP2D6), such as codeine, antidepressants and neuroleptics.The detection of allelic variants described as non-functional were useful to explain some circumstances of death in the study of tramadol positive cases and demonstrate the importance of this genetic tools to forensic toxicology and pathology.

TuOP 26 POSTMORTEM IDENTIFICATION OF METABOLIZER TYPE: A PROTEOMICS APPROACH

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Differences in xenobiotic metabolism rate between individuals can lead to fatal consequences. For example, a slow metabolizer can suffer a fatal intoxication due to the accumulation of the drug while taking a normal chronic dosage. There are two factors that determine metabolic capacity: genetics and expression. Genetic polymorphisms (mutations) of cytochrome P450 (CYP) metabolizing enzymes can have a severe impact on the enzymatic metabolizing efficiency. On the other hand, differences in biological factors such as disease, age and gender lead to varying CYP expression and therefore concentrations in the liver. Currently, metabolic capacity is assessed only through the genetic aspect by DNA sequencing.
The aim is to develop a novel approach for postmortem estimation of metabolic capacity encompassing both genetics and expression. This initial work focuses on CYP 2D6 and CYP 3A4, two of the most prevalent and important drug metabolizing enzymes. These liver proteins were targeted using LC-MS/MS to quantitate both the wild type ("normal") and mutated enzymes.
Liver homogenate was prepared by mechanically blending liver with 0.25 M sucrose, 150 mM NaCl, 50 mM Tris pH 7.0 in a 1:10 mass ratio. The liver microsomal fraction was pelleted through ultracentrifugation at 100 000 x g for 1 h, resuspended in digestion buffer (50 mM NH_4HCO_3, 4 mM DTT, 0.005% Brij-L23) and concentrated on a 10 kD centrifugal filter. Purified proteins were resuspended in digestion buffer, denatured at 95°C, alkylated with iodoacetamide and digested with 20 µg of trypsin or chymotrypsin at 37°C overnight. The digest was cleaned by solid phase extraction using Oasis mixed mode cartridges (Waters). The peptide extract was separated using an 18 minute step/ramp gradient (0.1% formic acid to acetonitrile) on a C18 column. Peptides were detected using MRM on an AB Sciex 5500 QTrap mass spectrometer and quantitated with recombinant CYPs (XenoTech) using a 6 level calibration curve with a dynamic range of 20 to 1000 pmol/g liver.
Peptides characteristic of normal CYPs and the most common and deleterious mutations (CYP 2D6*2, *4, *10, *17 and CYP 3A4*2, *3, *4, *5) were detected. Identification of these peptides enable assessment of genetic metabolic capacity potential. Expression level differences were evaluated using proteotypic peptides common to both normal and mutant CYPs. Preliminary results highlight large biological differences between individuals of up to 800% for

CYP 3A4 and 500% for CYP 2D6. Quantification results showed an intra-batch method reproducibility of 15%. An algorithm to estimate the metabolic capacity based on the LC-MS/MS results is being developed.
We have developed a method to estimate the metabolic capacity postmortem that takes into account not only genetics but, more importantly, differences in CYP expression level. This method yields a more precise and accurate estimate of the metabolic capacity than genotyping. This is an additional tool to help the forensic toxicologist to establish the manner of death.

TuOP 27 CANNABINOID PHARMACOKINETIC AND BEHAVIOURAL PROFILES AFTER DIFFERENT ROUTES OF ADMINISTRATION IN RATS

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Animal studies of cannabinoids usually employ parenteral routes of administration whereas in humans pulmonary (smoking/vaporized) or enteral administration is typical. Pulmonary administration of cannabis produces the greatest bioavailability of delta-9-tetrahydrocannabinol (THC), with serum concentrations peaking within minutes and subjective effects almost immediately. After both parenteral and enteral administration, subjective effects are delayed from 30 to 130 min and cannabinoid serum concentrations are significantly lower due to considerable first-pass liver metabolism, resulting in higher levels of psychoactive metabolite 11-hydroxy-delta-9-tetrahydrocannabinol (11-OH-THC). Cannabidiol (CBD) is known to modify the effects of THC by antagonizing some THC-effects, especially decreasing anxiety and psychotic symptoms.
The aim of this study was to compare subcutaneous, gastric and pulmonary (vaporized) routes of cannabinoid (THC, CBD and their combination) administration in rats. The parameters studied were: 1) pharmacokinetic profile of THC, CBD and 11-OH-THC in serum and brains within 24 hours 2) behavioural effects of cannabinoids in the open field and effects on sensorimotor processing after cannabinoid administration.
Male adolescent Wistar rats (cca 250 g b.w.) were exposed to THC, CBD and THC/CBD via (i) pulmonary administration of 20 mg/kg of each drug (vaporized for 5 min and inhaled by 4 animals in a group, delivered via Volcano® vaporizer); (ii) gastric probe (10 mg/kg) and (iii) subcutaneous (10 mg/kg). Following the treatment, the rats were sacrificed at 0.5, 1, 2, 4, 8, 24 hours after administration. Concentrations of THC, 11-OH-THC and CBD in serum and brain were determined by gas chromatography mass spectrometry (GC-MS). Locomotor activity after the drug administration was tested in the open filed test and the sensorimotor gating in the test of prepulse inhibition (PPI) of acoustic startle reaction.
The peak concentration of cannabinoids appeared at 1) 0-15 min after vaporization, 2) between 1-2 hours after gastric administration and 3) after 1 hour after subcutaneous administration. After oral administration of the THC/CBD mixture, brain THC concentrations almost doubled compared to THC administration alone and the concentrations were the highest from all routes of administration. In behavioural assays in the open field only gastric administration of THC showed significant effect on locomotor activity. CBD did not have any impact on this sedative effect. THC in all experiments disrupted the PPI, an effect that was only after subcutaneous administration partially normalized by CBD. CBD alone had no effect on locomotor activity, but had a transient disrupting effect on PPI.
The results of the present study for the first time describe the pharmacokinetics of subcutaneous administration which in contrast to pulmonary and gastric displays a slow continuous release of the drug to the organism with only a moderate peak of serum and brain concentrations. Robust accumulation of THC and 11-OH-THC after gastric administration in brain was observed due to the first pass metabolism. This is most likely the cause of the THC sedative effects. CBD co-administration increased brain concentrations of THC most likely due to the inhibition of liver metabolism. This work was supported by project MICR VG20122015080 and ED2.1.00/03.0078

TuOP 28 CONTROLLED VAPORIZED CANNABIS, WITH AND WITHOUT ALCOHOL: SUBJECTIVE EFFECTS AND BLOOD CANNABINOID DISPOSITION

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Increased medical and legal cannabis intake is accompanied by greater use of cannabis vaporization and more driving under the influence of cannabis cases. Despite frequent simultaneous Δ9-tetrahydrocannabinol (THC) and alcohol use, potential pharmacokinetic and pharmacodynamic interactions are not well understood.
The aim of this study was to evaluate blood cannabinoid disposition and subjective effects after vaporized cannabis, with and without simultaneous oral low-dose alcohol.
Adult cannabis smokers (≥1x/3 months, ≤3 days/week) drank placebo or low-dose alcohol (target ~0.065% peak breath-alcohol concentration [BrAC]) 10 min prior to inhaling 500 mg placebo, low-dose (2.9% THC), or high-dose (6.7% THC) vaporized cannabis (within-subjects, 6 alcohol-cannabis combinations). Blood was obtained and BrAC measured before and up to 8.3 h post-dose, and subjective effects measured at matched time points with visual-analogue scales.
Nineteen participants (13M, 6F, ages 21-37 years) completed all sessions. Median [range] maximum blood concen-

trations (Cmax) for low and high THC doses (no alcohol, first blood collection 10 min after smoking initiation) were 32.7 [11.4-66.2] and 42.2 [15.2-137] µg/L THC; 2.8 [0-9.1] and 5.0 [0-14.2] µg/L 11-hydroxy-THC (11-OH-THC); and 1.3 [0-3.4] µg/L and 1.1 [0-3.4] µg/L cannabinol (CBN). With alcohol, low and high dose Cmax were 35.3 [13.0-71.4] and 67.5 [18.1-210] µg/L THC; 3.7 [1.4-6.0] and 6.0 [0-23.3] µg/L 11-OH-THC; and 1.8 [0-4.2] µg/L and 1.6 [0-5.3] µg/L CBN, significantly higher than without alcohol. Other blood cannabinoids' concentrations did not significantly differ with alcohol. Cannabinoids 11-nor-9-carboxy-THC (THCCOOH), THCCOOH-glucuronide, THC-glucuronide, and cannabidiol (CBD) low and high dose Cmax without alcohol were 14.5 [4.4-84.2] and 23.8 [2.6-66.6] µg/L THCCOOH; 25.9 [0-213] and 48.2 [0-145] µg/L THCCOOH-glucuronide; 0 [0-0] and 0 [0-0.8] µg/L THC-glucuronide; 0 [0-0] and 1.0 [0-3.6] µg/L CBD. Subjective effects persisted through 3.3-4.3h, with alcohol potentiating cannabis effects' duration. Effect-versus-THC concentration and effect-versus-alcohol concentration hystereses were counterclockwise and clockwise, respectively. With detection cutoff THC ≥1 µg/L, ≥16.7% of participants remained positive 8.3 h post-dose; whereas ≤21.1% were positive at 2.3 h with THC ≥5 µg/L.
Vaporization is an effective THC delivery route. Vaporized cannabis subjective effects were similar to those previously reported after smoking, with duration extended by concurrent alcohol. The significantly higher blood THC and 11-OH-THC Cmax with alcohol possibly explain increased performance impairment observed from cannabis-alcohol combinations. Chosen THC detection cutoffs should be considered carefully to best reflect performance impairment windows. These results will help facilitate forensic interpretation and inform the debate on drugged driving legislation.

TUOP 29 GHB AND ITS NEWLY DESCRIBED METABOLITE GHB-β-O-D-GLUCURONIDE – DISCRIMINATION EXOGENOUS/ ENDOGENOUS VIA LC/MS/MS AND GC/C/IRMS

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Due to the short detection time of GHB, the verification of its intake is often problematic. Recently, a glucuronid-metabolite of GHB (GHC-GLUC) has been identified in urine and was proposed to have a longer detection period. The aim of this study was to describe endogenous concentrations of GHB-GLUC in urine and plasma and compare these to concentrations after the intake of GHB in a clinical trial. Additionally, urine samples were measured by gas chromatography/combustion/isotopic ratio mass spectrometry (GC/C/IRMS) to examine if this is a suitable method for the distinction between endogenous and exogenous GHB and an enlargement of the detection window.
Urine samples (n=28) and plasma samples (n=13) of control subjects were only measured for endogenous GHB-GLUC concentrations. Furthermore, we received serum and urine samples of a patient therapeutically taking 2,25 g Xyrem® (GHB). The patient abstained from GHB intake for 3 days before the treatment. Samples were collected prior to and at different times after the intake (0.5 h, 1 h, 1.5 h, 2 h, 4 h, 6 h, 8 h, 12 h, 24 h, 48 h, 72 h) and were analyzed with a LC/MS/MS system operated in multiple reaction monitoring mode. Chromatographic separation was achieved using a HILIC column for GHB and a HYPERCARB column for GHB-GLUC. The urine samples were also analyzed by GC/C/IRMS. The analytes were separated by preparative HPLC and a Agilent Eclipse XDB-C18 column was used.
Endogenous GHB-GLUC was detected in concentrations ranging from 0.2 µg/ml to 7,9 µg/ml (mean: 1.9 µg/ml ± [3SD] 5.4 µg/ml; n=28) and 2.7 ng/ml to 47.9 ng/ml (mean: 18.4 ng/ml ± [3SD] 39.9 ng/ml; n=13) in urine and plasma respectively. Urine samples collected after the single intake of 2,25 g GHB, showed a time-dependent increase of GHB and its metabolite GHB-GLUC. While GHB concentrations in urine decreased to endogenous concentrations (cut-off < 10 µg/ml) after 4 hours, elevated concentrations of GHB-GLUC could be determined up to 8 hours after intake. In serum samples the last GHB concentration above the cut-off-value (> 5 µg/ml) could be measured 2 hours after the intake. However, a time-dependent increase of GHB-GLUC concentrations could not be detected. The results of the GC/C/IRMS were as follows: The administered GHB itself was found to have a 13C/12C ratio of -28,64 ‰,while for endogenous GHB, a 13C/12C ratio of -23,66 ‰ was detected in the urine sample of the patient prior to the administration. Six hours later, the 13C/12C ratio was found to be -25,72 ‰. Therefore, an exogenous content could still be assumed.
A pharmacokinetic profile for GHB and GHB-GLUC was generated. The inclusion of GC/C/IRMS does not increase detection windows.

TUOP 30 BEHAVIOURAL AND PHARMACOLOGICAL CHARACTERIZATION OF A NOVEL CANNABIMIMETIC ADAMANTANE-DERIVED INDOLE, APICA, AND CONSIDERATIONS ON THE POSSIBLE MISUSE AS A PSYCHOTROPIC SPICE ABUSE, IN C57BL/6J MICE

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The novel adamantane derivative APICA (N-(adamtan-1-yl)-1-pentyl-1H-indole-3-carboxamide) was recently identified as a cannabimimetic indole of abuse. Despite its novel structure, APICA recalls cannabimimetic indoles, such as representative member JWH-018. This emerging abuse problem, together with the paucity of information about the bioactivity of APICA emphasizes the need for further evaluation of the in vivo pharmacology of this novel indole-derived compound.
Behavioural and pharmacological characterization of APICA and considerations on the possible misuse as a psychotropic spice abuse
In the present study, the effects of APICA (0 - 1 - 3 mg/Kg, i.p.) were tested in C57BL/6J mice, in a battery of tests that are sensitive to the effects of psychoactive cannabinoids, including body temperature; locomotor activity and behavioural reactivity in the open field test; nociception in the tail flick assay; motor coordination in the accelerating Rotarod; recognition memory in the novel object recognition test. Furthermore, the highest dose was also evaluated following the pre-treatment with the CB1 antagonist AM251 (3 mg/Kg, i.p.) or the CB2 antagonist AM630 (3 mg/Kg, i.p.).
Our results show that APICA was able to dose-dependently decrease locomotor activity and behavioural reactivity in the open field, whereas only the highest dose was able to induce hypothermia, analgesia, motor ataxia and recognition memory impairment, with respect to vehicle ($p<0.01$; $p<0.001$). The pretreatment with the CB1 antagonist AM251 elicited an increase in body temperature, total distance travelled in the open field, descent latency in the Rotarod, and a decrease in tail flick latency ($p<0.05$; $p<0.01$). On the other hand, pretreatment with AM630 did not induced significant differences, resulting in hypothermia, antinociception and motor ataxia, with respect to vehicle.
This study supports preliminary reports on APICA cannabimimetic properties, extending its detrimental effects on cognitive function. Moreover, these properties can be attributed to the CB1 receptor activity, indicating APICA as a selective CB1 receptor agonist.
APICA as a selective CB1 receptor ligands may be useful in the pharmacotherapy of several pathological conditions; nevertheless, the increased use as designer drugs of abuse raises significant public health concerns.

WEDNESDAY, September 2nd

8.30-10.30 am SESSION 1 – Driving Under the Influence II

WOP 1 COMPARISON OF ILLICIT DRUG CONCENTRATIONS IN PLASMA AFTER ROADSIDE TESTING IN URINE OR ORAL FLUID IN THE BELGIAN DRIVING POPULATION

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Title must be adapted see reviewers comments (see e-mail send on 30/04) Legislators worldwide want to improve traffic safety by creating law enforcement procedures detecting driving under the influence of drugs (DUID). These procedures commence with an observation of external signs of impairment or suspected recent drug use by a police officer. Afterwards, on-site urine or oral fluid (OF) immunological tests can be applied to apprehend drugged drivers. Commonly final judicial measures are only taken after confirmation of these on-site screenings by a chromatographic analysis in blood/plasma or OF.
Comparing drug plasma concentrations obtained from two legal DUID procedures, with differences in police observation techniques, on-site screening tests (urine versus OF), and the analytical cut-off concentrations in plasma. Plasma data related to positive on-site tests were compared: urine screenings using Dipro Druglab Panels gathered from April 2008 to September 2010 (set 1; n= 4109), OF tests using Securetec Drugwipe 5+ gathered from October 2010 to April 2013 (set 2; n= 3917), and an extra set of OF data collected during 2014 with an adapted immunological properties for Δ9-tetrahydrocannabinol (THC) and cocaine (set 3; n= 2698). Plasma confirmation was performed by validated gas or liquid chromatographic methods. False positives (FP) were defined as the number of non-confirmed screening results. Statistical analysis was done using IBM SPSS Statistics 21.
The percentage of plasma samples of tested drivers, in which none of the positive screened target drugs were present in a concentration above the legal cut-off value, has decreased from 17% to 7% since the introduction of the current legislation involving OF screening. The percentage of THC positive screenings is diminished from 88% using urine tests to 66% when using OF tests. However, a significantly higher ($p<0.001$) median plasma THC concentration was observed after OF screening (8.5 ng/mL) compared to urine screening (6.0 ng/mL) indicating more recent use. Moreover, the number of FP dropped from 25% to 9%. When evaluating set 3, the FP decreased further to 6%. The observed median THC plasma concentration did not significantly differ between the two OF sets ($p=0.063$). For cocaine no changes in FP were observed between the groups. The median cocaine plasma concentration decreased from 47 to 34 ng/mL using the adapted OF device ($p<0.001$). In set 3 more plasma samples contained only benzoylecgonine (but no cocaine). However, no significant difference was observed ($p=0.073$). The amphetamine FP were respectively 31% and 25% when using urine or OF tests. For opiates the FP dropped from 72% using urine screening to about 52% when using OF screening.
By changing the on-site drug screening procedure from urine to OF and lowering the cut-off values for confirmation in plasma, a better approach towards DUID was developed. Even with a limited pre-selection of drivers, the results demonstrate a lower percentage of FP for cannabis, amphetamine and opiates. Especially for cannabis it is undoubtedly demonstrated that this approach leads to a detection of recent drug use.

WOP 2 APPLICATION OF A LC-MS/MS METHOD FOR SELECTED SYNTHETIC CATHINONES AND PIPERAZINES TO DRIVERS' ORAL FLUID SPECIMENS

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Spanish drivers have to undergo on-site drug testing in oral fluid(OF) for preventive drug controls, including the detection of cocaine, opiates, cannabis, and amphetamine derivatives. However, in the last years, new unregulated drugs of abuse, including synthetic cathinones and piperazines, have emerged into the drug market to substitute illegal drugs, which cannot be identified with the current methodologies.
To develop a LC-MS/MS method for the determination of methedrone, mephedrone, methylone, 3,4-methylenedioxypyrovalerone(MDPV), fluoromethcathinone, fluoromethamphetamine, 1-(3-chlorophenyl)piperazine(mCPP) and 3-trifluoromethylphenylpiperazine(TFMPP) in OF. To analyze OF specimens collected on the roadside to evaluate the presence of these new synthetic drugs among drivers.
0.5 mL OF were extracted using the same solid phase extraction(SPE) procedure employed in our laboratory for the determination of cocaine, opiates, cannabis, and amphetamine derivatives. In addition, the two analytical methods also shared the same chromatographic column and mobile phases, so that two injections of the same SPE extract allow the determination of the classical and new drugs of abuse. Method validation included linearity, limits of detection(LOD) and quantification(LOQ), endogenous and exogenous interferences, imprecision, accuracy and matrix effect in neat and Quantisal® OF, extraction recovery and recovery from the Quantisal®, dilution integrity and stability at different conditions. The method was applied to OF drivers' specimens sent to our laboratory in 2013 and 2014 to confirm positive on-site results for cocaine, opiates, cannabis, and/or amphetamine derivatives.
The method was specific, and linearity was verified between 0.2-0.5 to 200 ng/mL. LOD and LOQ were 0.025-0.1 ng/mL and 0.2-0.5 ng/mL, respectively. Imprecision and accuracy in neat and Quantisal® OF were %CV=0.0-12.7% and 84.8-103.6% of target concentration, and %CV=7.2-10.3% and 80.2-106.5% of target concentration, respectively. Matrix effect in neat and Quantisal® OF were -11 to 399.7% and 79.6 to 107.7%, respectively. Accuracy after dilution

was 75-99% of target concentration, and stability studies showed -14 to 30.8% loss. The method was applied to 1454 drivers' OF specimens that were positive for one or several classical drugs. Twenty-five (1.7%) specimens tested positive for the new drugs; however, mCPP due to trazodone prescription was detected in three cases; therefore, 22 (1.5%) specimens were positive due to a recreational use of the cathinones and/or piperazines. Amphetamines, cocaine and THC together were confirmed in 12 of the positive cases (54.5%), and at least two classical drugs were present in 81.8% of them. A cathinone (methylone) was identified in only one out of the 184 on-site false positive cases to amphetamine derivatives. Regarding the analytes detected, 8 specimens were positive for methylone (3.7-10518.9 ng/mL; mean=2558.2 ng/mL; median=283.6 ng/mL), 8 for mCPP (1.4-242.9 ng/mL; 78.1 ng/mL; 51.5 ng/mL), 4 for MDPV (7.1-63.0 ng/mL; 23.2 ng/mL; 11.4 ng/mL), 2 for mephedrone (4.2-8.8 ng/mL; 6.6 ng/mL; 6.6 ng/mL), and 1 for TFMPP (10.2 ng/mL).
A LC-MS/MS method was developed and validated for the determination of selected synthetic cathinones and piperazines in OF. Analysis of a significant number of drivers' OF specimens revealed the presence of these new drugs of abuse.

WOP 3 ULTRA-RAPID TARGETED ANALYSIS OF 40 DRUGS OF ABUSE IN ORAL FLUID BY LC-MS/MS

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The number of oral fluid specimens collected by the Road Policing authority in Victoria from random drug testing has greatly increased in recent years. Many thousands of oral fluid specimens are now confirmed in our laboratory for a number of drugs as per legislative requirements. Traditional chromatographic methods which have employed long analysis times (>20mins/sample) have needed to be improved in order to meet the volume of casework.
The aim of this study was to develop an LC-MS/MS targeted oral fluid analysis that covered a broad range of basic and neutral drugs of abuse (including other impairing drugs) by combining small sample volume, utilising simple extraction procedures and improved LC-MS/MS analysis and automated data processing.
After liquid-liquid extraction of 0.2 mL neat oral fluid or oral fluid in 0.1M phosphate buffer solution (collected via roadside screening via the Quantisal™ device), 40 basic and neutral drugs of abuse including amphetamines, benzodiazepines, cocaine and major metabolites, opioids, cannabinoids and some designer stimulants were separated using a Shimadzu Nexera X2 UPLC system with a C18 separation column (Kinetex C18, 4.6 × 50 mm, 2.6µm) using gradient elution with a mobile phase of 50mM ammonium formate buffer (pH 3.5) / acetonitrile over 5 minutes (0.5 min re-equilibration time). The drugs were detected using an ABSciex® API 4500 Q-TRAP LC-MS/MS system (ESI + MRM mode, three transitions per analyte). The method also monitors C13 isotopes of MDMA and methamphetamine to combat detector saturation effects. The method was fully validated in accordance with international guidelines. Matrix effects and extraction efficiency were acceptable with most analytes showing >80% response, with low variation (within 20% of mean response). The method was linear from 1-50ng/mL. Precision, accuracy and repeatability were satisfactory for all analytes investigated with relative standard deviations of approximately 6% or better. Furthermore, utilization of C13 isotopes for confirmation of methamphetamine and MDMA ion ratios has enabled qualitative reporting of drug presence above nominated cut-offs in cases far exceeding the C12-calibration range, reducing the need for re-analysis with dilution. The method described has been successfully applied for analysis of over 3000 oral fluid specimens received as part of law enforcement procedures collected at the roadside in Victoria.
The fast and reliable extraction method combined with rapid LC-MS/MS analysis of a broad range of drugs of abuse and automated data processing gives the opportunity for greater throughput, decreased turnaround times and reduced analysis cost for forensic and clinical toxicology.

WOP 4 TIME LAPSE PERIOD OF POSITIVITY OF ORAL-FLUID TEST IN CHRONIC AND OCCASIONAL CONSUMERS AFTER ADMINISTRATION OF 10 OR 30 MG OF SMOKED THC VERSUS PLACEBO; CORRELATION WITH BLOOD CONCENTRATIONS

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No studies have been made to date to evaluate the period of detection time for the oral fluid tests Drugwipe 5S in paralell with the concentration of blood with regards to the amount consumed and the type of consumer
A crossover, randomized, double blind study using a dose of 10mg or 30 mg versus placebo of smoked THC over three periods each separated by a one week wash-out period was carried out
15 occasional consumers (1-2joints/week) and 13 chronical consumers (1-2 joints/day), validated using a capillary measurement of THC, cannabinol and cannabidiol, were included after written consent. Before each test period, each volunteer was submitted to a saliva screen test for drugs of abuse that had to be necessarily negative and a blood sample was taken. Each volunteer then consumed its joint (15 inhalations each 40s during 10 min). The THC remaining in each joint stub was quantified. Alveolar CO before and 30 minutes after smoking was measured (characterizing the importance of the inhalation). A blood sample was taken 5, 15, 30 minutes, 1, 2, 4, 6, 8, 10, 12 and 24 hours after the end of the joint, and a saliva test was carried out a the same time.
773 tests were performed. Three false negatives results were observed, one chronic smoker being negative during the 24h after administration of the 30mg-dose. 4 occasional smokers presented false positives results always at T5 minutes after the administration of the placebo, whereas they were also 4 patients in the chronic group but for longer, until T2h. Two chronic volunteers became again positive 24 hours after smoking their joints without having re-consumed in the meantime. The positive time lapse after administrating both the weak and medium doses was not significantly different either with the occasional or chronic smokers. The time lapse is variable, taken between 15 minutes (observed

with 3 occasional smokers after having absorbed the weak dose) and 8 hours after smoking the joint except for one chronic smoker who remained positive 12 hours after having smoked the 30mg-dose. No correlation with blood concentration could be established. The tests could be positive with a blood concentration of THC 0.1 ng/mL with the occasional volunteers whereas they could be negative with a blood concentration of 5 ng/mL with the chronic volunteers No difference was found between the occasional or chronic smokers concerning the positive time lapse of the saliva tests in the normal conditions of use by the Police force and this, irrelative of the dose absorbed. The time-lapse period of positivity is between 15 minutes and 12 hours depending on the volunteer. No relationship can be made between time-lapse period of positivity and the blood concentrations

WOP 5 OPTIMIZATION OF AN AUTOMATED SOLID PHASE EXTRACTION TO DETERMINE DRUGS IN PRESERVED ORAL FLUID USING ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Driving under the influence of drugs (DUID) not only increases the risk of accidents, but also the severity of the accident and of the caused injuries. To increase public awareness of DUID and road-safety, 'per se' legislations were set-up worldwide. Oral fluid (OF) has been investigated for both screening and confirmatory purposes in DUID context, because of its ease of sample collection at the roadside. In Belgium for example, police officers no longer apply urine but OF screening tests. In the near future, the current blood confirmation analysis will become second choice, as OF samples will be collected to confirm recent drug use. Consequently, chromatographic mass spectrometeric methods to quantify the analytes of interest in OF are required. UPLC-MS/MS has become a powerful tool for high-troughput confirmation of multiple illicit drugs in OF. Since biological samples are usually not directly compatible with UPLC-MS/MS, efficient sample pretreatment is required. For this purpose, commercially available workstations can be introduced for laboratory automatisation.
The aim of this work was to automate a sample preparation procedure extracting amphetamine, MDMA, morphine, 6-MAM, cocaine and benzoylecgonine from preserved oral fluid samples collected with the Quantisal® device.
Samples were extracted by solid phase extraction with cation exchange cartridges (Waters Oasis® MCX 3cc, 60 mg) using a TECAN Freedom Evo 100 base robotic system. Block modules were carefully selected using the same consumables as for the manual procedures, reducing costs and/or manual sample transfers. The present configuration also includes a pressure monitoring pipetting system increasing pipetting accuracy and detecting sampling errors. The TECAN was used for the complete sample preparation procedure, including the preparation of the calibration curve and addition of the internal standard. Only evaporation of the eluate was performed offline, to save time and to ensure sample stability. Sample integrity was guaranteed since the extracts were eluted directly in LC vials.
Following extraction, compounds were separated using a BEH C18 analytical column on a UHPLC-MS/MS system. All the compounds eluted in less than 3.2 min.
Intra- and interday relative standard deviations (RSD's) were within 10%, while bias was within ±13%. Recovery ranged from 57.5 to 94.0 % (RSD's from 2.5 to 19.4 %) and matrix effects were lower than ±18.9 % (RSD's from 1.2 to 5.3%). The limits of quantification ranged from 2.5 to 12.5 ng/mL and were in accordance with the Belgian law concerning DUID. No cross-contamination in the automated SPE system was observed after the analysis of samples spiked with 10 times the upper limit of quantification.
To conclude, automation resulted in acceptable precision and accuracy and in minimal operator intervention, leading to safer sample handling and less (technical) time-consuming procedures. This method will be applied on OF samples collected from drivers suspected from DUID.

WOP 6 USE OF PSYCHOACTIVE SUBSTANCES AMONG TRUCK DRIVERS IN THE HIGHWAYS OF THE STATE OF SAO PAULO, BRAZIL

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Traffic accidents are responsible for approximately 1.2 million deaths per year worldwide. In Brazil, there were more than 44,000 traffic-related deaths in 2014. The use of psychoactive substances while driving is considered a major contributing factor for the occurrence of these accidents. In addition to alcohol, the most used illicit drugs in our country are amphetamines, cocaine and cannabis. Amphetamines and cocaine are used by some truck drivers to combat the detrimental effects of fatigue in cases they have to drive for many hours in a row. Therefore, it is necessary to better understand this problem in order to help authorities for the implementation of public policies related to drug use by truck drivers in Brazil.
The objective of this work was to evaluate the prevalence of psychoactive substance (amphetamines, cocaine and cannabis) use among truck drivers in the highways of the State of Sao Paulo through toxicological analysis in urine and to correlate the results with sociodemographic and occupational data.
This is an observational cross-sectional study in which data collection was carried out between 2008 and 2012. This study included 1,316 drivers who were randomly stopped by the police (not under investigation for suspected DUI). The volunteers provided a urine sample after signing a consent form and answered a questionnaire with sociodemographic and occupational data. The urine samples were analyzed by immunoassay and gas chromatography-mass spectrometry. Of the total samples collected, 7.8% (n = 103) were positive for one or more tested drugs and/or its metabolites,

with 3.4% positive for amphetamine, 2.8% for cocaine and 1.1% for cannabinoids. The remaining 0.5% corresponded to cases with more than one drug. The three drugs were found during almost the whole studied period, except in 2008. Toxicological findings were distributed differently according to some variables. Age, employment period and marital status were associated with drug use, while the employment type, ethnicity and education were not. Travel length and night resting period were also associated with drug use. Daytime resting period, travel length period, driving time without rest, number of occupants and freight content did not correlate significantly with drug use. However, the association between alcohol use (reported by truck drivers) and drug use was found.
The results indicate that the use of psychoactive substances by truck drivers is common and this use is associated with age, employment period and marital status, as well as distance traveled and night resting period.

WOP 7 CONFIRMATORY AND COMPARATIVE DRUG ANALYSIS OF ORAL FLUID / BLOOD SPECIMENS FROM DRIVERS

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The oral fluid (OF) became a widely used sample for preliminary screening of drugs of abuse, particularly in on-site (roadside) drivers' testing. The confirmatory drug analyses of OF specimen have to be performed, especially if the initial screening is based on immunosorbent assay and no other sample (blood, urine) is submitted for toxicology testing. However, the correspondence of analytical results for OF / blood is important for the forensic interpretation.
In the present study we report results of confirmatory on-site / in-lab analysis of OF samples as well as comparison between preliminary on-site OF tests with systematic toxicological analysis (STA) of the corresponding blood sample.
A fast and simple sample-preparation procedure for GC-MS detection of illicit drugs in OF specimen collected from roadside testing devices (Dräger Drug Test 1200 / 5000 cartridge) was used. The cartridge' test strips were used and an extraction with methyl tert-butyl ether (3 mL) was performed in the presence of 1 M NaOH in ethanol (100 µL) of targeted compounds. After sonication (3 min), the extract was separated and concentrated under nitrogen up to 50 µL.
The detection of drugs was performed by GC-MS. The results obtained are compared with those of parallel analysis of blood samples sent for conformational analyses (N = 50; enzyme hydrolysis/LLE or SPE/derivatisation/GC-MS).
The laboratory testing of used on-site devices (N=50) shows 69% confirmation with the preliminary roadside result. In the other 31% cases some differences were noticed, but at the same time some preliminary factors (as pre-lab storage period and storage conditions) were unclear. The comparative study with STA blood testing shows an increased number of mismatching results due to cross-reactivity of the OF-device for amphetamine, methamphetamine and 3,4-methylenedioxy-methamphetamine (28% of positive tests for amphetamine type stimulants), and for opiates (33% of positive opiates tests). The best corresponding results between on-site OF and in-lab blood analysis were registered for THC (89% of THC-positive tests). 70% of the positive preliminary OF on-site testing devices results were confirmed by blood testing.
The results assumed that confirmatory analyses of OF still have to be performed in all cases where positive result from roadside testing is obtained. In-lab analysis of OF should provide an assessment for specificity of the screening devices. The data obtained showed a relatively good correlation between tested positive OF-samples. However, a significant cross-reactivity was registered in cases when the illicit drug is not a pure substance and co-administered with similar impurities (amphetamines, opiates).

WOP 8 ASSOCIATION BETWEEN ELEVATED HAIR ETG AND ALCOHOL RELATED TRAFFIC DEATHS

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Drunk-driving is one of the most important risk factor of road accidents. However, if there is a widespread consensus on the correlation of the degree of impairment of the driving ability with the blood alcohol concentration (BAC), a much weaker evidence can be found in the current literature on the correlation between the chronic abuse of alcohol and an increased risk of causing traffic accidents.
Aim of the present work was to evaluate the association of hair EtG and the occurrence of alcohol related fatal road accidents, in the frame of the "Safe drive" project.
The study was carried out on 70 subjects died in car accidents (Group A). In each case, samples of blood, vitreous humour and hair were collected for the determination of blood alcohol, psychotropic drugs and EtG, respectively. The group A was divided into two subgroups on the basis of the BAC legal limit adopted in Italy (BAC ≤0.5 g/L: A1; BAC >0.5 g/L: A2). The control group (Group B) included 70 subjects taken from the general population holding the driving licence without a traffic accident in their recent history. The determination of BAC in blood was performed by HS-GC, whereas EtG in hair was analysed by NCI-GC-QQQ-MS. The drug screening was performed by immunoassay. The EtG data were analyzed using the cut-off of 30 pg/mg proposed by SoHT.
The drug screening performed on vitreous humour samples identified 20 cases positive for one or more classes of psychotropic drugs. These cases were excluded from the study, which so included 50 fatal cases. The preliminary results showed 18% of cases with elevated hair EtG (> 30 pg/mg) in subjects with BAC ≤0.5 g/L (subgroup A1) and 44% of cases with elevated hair EtG in subjects with BAC > 0.5 g/L (subgroup A2). The difference between subgroup A1 and subgroup A2 was significant at the Fisher's Exact Test even if with only a $p < 0.05$. The analysis of the samples of the control group is in progress.
The present data suggest a significant difference between hair concentration of EtG in alcohol related and non-alcohol related traffic accidents. The analysis of the controls will allow for the calculation of the Odds Ratio offering a stronger tool for interpreting in a prospective way the hair EtG concentration in function of the risk of alcohol related traffic accidents.

WEDNESDAY, September 2nd

11.00-12.45 pm SESSION 2 – Poisoning Case Reports

WOP 9 DATURA AND HALLUCINATIONS: THE ATROPINE RISK

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Datura belongs to the family of Solanaceae and is well known for its hallucinogenic properties. All of its parts contain atropine, and scopolamine, which may induce anticholinergic syndrome after ingestion by antagonism of central and peripheral muscarinic receptors
We report the case of a 27 year-old man admitted to an Emergency Unit for coma after a voluntary datura self-poisoning. Clinical examination revealed bilateral and reactive mydriasis. He is generally hypotonic. He is also hypoxemic in ambient air. He alternates between unconsciousness and wake phases. He holds incoherent, aggressive and has hallucinations. The patient has no tachycardia or hyperthermia. There is no clinical bladder distension. However, catheterization globe brings 600 mL of urine output immediately. His girlfriend says to medical doctors he has chewed leaves of datura, purchased on internet. It's a voluntary festive datura self-poisoning.
Identification and quantification of alkaloids were performed using a validated method using LC-MS / MS on plasma and urine samples collected respectively 8 and 18 hours after intoxication. The samples were extracted by hexane / ethyl acetate (1/1, v / v). After evaporation of the organic phase, the residue is taken up in 80 µL of mobile phase. Ten µL were injected into the chromatographic system. The analytes were separated on a Hypersil GOLD PFP column with a gradient mobile phase consisting of a mixture of acetonitrile and 0.1% formate buffer. The detection was performed in MRM mode on a mass spectrometer LCQ TSQ Vantage XP (Thermofisher) after ionization in positive ESI mode. Two transitions were selected for the identification and quantification of atropine, scopolamine and ketamine-d4, the internal standard used for the analysis. Method was full validated according to EMEA recommendations.
The analysis of blood samples collected 8 hours and 18 hours after datura consumption showed scopolamine concentrations at 1,2 ng/mL and 0,6 ng/mL, respectively and confirmed acute datura poisoning. At the same time, the urinary levels of scopolamine were 132 ng/mL and 71 ng/mL. Atropine was not found in any sample. Scopolamine induces peripheral and central anticholinergic manifestations developed by the patient. The absence of atropine in the samples could be associated to the consumption of Datura inoxia, a species of datura without atropine.
Consumption of datura for recreational purposes is not uncommon because of its hallucinogenic effects. It often leads to acute poisoning with favorable outcome. The low plasma concentrations and short elimination half-life of these alkaloids can lead to delayed diagnosis if their research and their quantification by a specific method were not prescribed. It seems important to inform primary care physicians about this abuse, especially in cases of agitation and confusion in child, adolescents or young adults.

WOP 10 SUICIDE OF A NURSE IN A HOSPITAL ENVIRONMENT INVOLVING ANESTHETIC DRUGS

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Toxicological postmortem analyses can be difficult to achieve when the decedent is working in a medical environment. Numerous anesthetic drugs can be used to commit suicide and there is a need to implement a specific strategy, particularly when the amount of specimens is limited.
Develop an analytical strategy to test the biological specimens in case of suicide involving a nurse.
Miss P., a nurse was found dead. Taking into consideration her profession and her potential access to various controlled substances, a complete strategy devoted to the identification of medicinal agents used in anesthesia was organized. A general screening of the blood was achieved by liquid chromatography on a column C18 ACQUITY HSS after alkaline extraction by a mixture dichloromethane/isopropanol/n-heptane (25/10/65, v/v/v) and detection by tandem mass spectrometry (UPLC-Quattro Micro WATERS). The identification and the dosage of the propofol was made after introduction in headspace, followed by a separation on column DB-WAX (30m x 0.25 mm) and a detection by mass spectrometry on a FocusGC+DSQII (Thermo). After an initial screening of curare by LC-MS/MS (extraction by ions pairing), laudanosine was simultaneously identified by LC-DAD on a Symmetry®C8 column and quantified by gas chromatography coupled with mass spectrometry after separation on a HP5MS capillary column. The forensic investigations performed on postmortem peripheral blood identified the simultaneous presence of midazolam at 32.8 µg/mL, amiodarone at 238 µg/mL, propofol at 223 µg/mL, and finally laudanosine (the circulating form of atracurium or cisatracurium) at 915 ng / mL. Tests for other drugs and ethyl alcohol were negative.
All the concentrations of the compounds found in the blood of Miss P. were well above the usual therapeutic concentrations. Each concentration can be considered as potentially fatal. This multi-drug intoxication has probably produced a coma, complicated by respiratory failure and cardiovascular collapse (hypotension, cardiogenic shock) leading to the death of Miss P. The forensic discussion will focus on the characteristics of the molecules used in anesthesiology and emergency service on their availability, their regulation and finally their consumption patterns

WOP 11 POISONOUS PLANTS AND ANCIENT HUNTERS: AN ANALYTICAL INVESTIGATION INTO THE PRESENCE OF PLANT ALKALOIDS ON HUNTING TOOLS FROM INTERNATIONAL MUSEUM COLLECTIONS

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Many archaeologists believe that our early ancestors used poison to kill or incapacitate their prey using commonly found plants. It is believed that the hunting tools were dipped into a paste of the plant material containing alkaloids or cardenolides which in turn was used to aid in the process of hunting. Until now, very little scientific evidence has been available to support this claim.
The aim of this project is to analyse arrow heads and other hunting tools from international museum collections, for the presence of plant poisons by mass spectrometry and hyphenated techniques. It is also to initially establish if it is possible to detect the plant alkaloids after thousands of years and to consider the implications of the findings in relation to sample preparation/interpretation of results.
Plant standards were provided by Alnwick Gardens, Northumberland. A sample of curare was provided by the Pitt Rivers Museum (Oxford). Swabs of archaeological hunting tools have been provided by the Museum of Archaeology & Anthropology (Cambridge), the Pitt Rivers Museum (Oxford), and the Museo Etnografico Pigorini of Roma (Italy). LC-MS or MS analysis was carried out on the extracts from the swabs in comparison to the plant standards.
Results have shown that it is possible to detect aconite in a pot 125 years old as well as alkaloids of the Strychnos species on arrow heads and darts swabbed in the museums. Current work includes the analysis of an arrow dating from 4000 BC, provided by the Phoebe A Hearst Museum of Berkeley (USA). Even though items were from the same collection, plant poisons were not found on all items when the same preparation and analysis technique were used. No information was provided with regards to where the samples were originally found or with respect to storage/cleaning therefore it is possible that the items may have come into contact with other poisoned items in the museum.
It is possible to detect plant poisons on hunting tools using MS and hyphenated chromatographic techniques. This work continues in the analysis of other archaeological artefacts from international museums.

WOP 12 A HIDDEN KILLER?: PREGABALIN IN FATALITIES

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The GABA analogue, pregabalin has become more widely prescribed and abused in recent years but is still not always included in laboratory analysis. Consequently, comparative data for interpretation are relatively limited.
The aim was to incorporate a method for analysis into laboratory protocols and assess the presence and concentration of pregabalin in casework and monitor other drugs used to assist determining toxicological significance in fatalities.
Blood concentrations of pregabalin measured using a specific LC-MS-MS method were recorded in post-mortem cases and drug driving, along with the presence of other drugs.
Pregabalin was detected in 93 post-mortem cases, 80 of which were drug related deaths. The concentrations found (median; range) were: drug deaths (7.0; <0.6-21.6 mg/L), alternative causes of death (2.6; <0.6-4.9 mg/L), fatalities where pregabalin is likely to have contributed to death (57.0; 28-182 mg/L). As an in life comparator, concentrations in drug driving cases (n=4) were: 2.8 median; 1.1-46.7 mg/L). Other drugs or alcohol were detected in all of the cases and the most common drug types (in decreasing frequency) were: antidepressants, opioids, benzodiazepines, opiates, alcohol, antipsychotics, cocaine, cardiac drugs, amphetamines, cannabis, anticonvulsants and antihistamines. New psychoactive substances (methoxphenidine and synthetic cannabinoids) were only found in two cases.
The results provide further data to assist in evaluating the significance of post-mortem pregabalin concentrations. Pregabalin, especially with concomitant use of other CNS depressant drugs, presents a significant toxicological risk and existing laboratory protocols should be checked for their suitability to detect pregabalin.

WOP 13 ETHYLPHENIDATE IN POST MORTEM CASES

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Ethylphenidate is a product of pro-drugs ethanol and methylphenidate. Ethylphenidate is a potent psychostimulant that acts as both a dopamine and norepinephrine reuptake inhibitor. Ethylphenidate may be formed when large quantities of ethanol and methylphenidate are co-ingested, via hepatic trans-esterification or, as in these cases, is administered as a parent compound. Ethylphenidate was made a controlled substance in the UK in April 2015. The UK National Poisons Information Service has limited experience of reported exposures to ethylphenidate. These exposures have not been confirmed by laboratory analysis. Clinical features reported are consistent with those of stimulants. The onset of effects appears to be within 45 minutes of exposure with user-reports suggesting the duration of action as 2 to 3 hours. There is a single peer-reviewed case report that records death may have had some contribution from, but was unlikely to have been caused specifically, with an ethylphenidate in femoral blood at a concentration of 110ng/mL.
To review the toxicological findings and circumstances of death in cases in which ethylphenidate was detected.
All routine cases underwent ethanol quantitation by headspace GC-FID, and screening / quantitation of matrices

by LC-MSMS. In the seven cases in which ethylphenidate was detected by screening, quantitation was performed by a validated LC-MS/MS method using deuterated methylphenidate as an internal standard. Cause of death was obtained from pathologists report.
Between February 2013 and January 2015 there were 7 cases in which ethylphenidate was detected. Methylphenidate and metabolite (ritalinic acid) were not detected in any of these cases (limit of detection 5ng/mL). Two cases had post mortem blood ethanol detected (30 and 74mg/100mL). Death in one case was concluded to have been due to ethylphenidate toxicity with a post mortem blood concentration of 2182 ng/mL. No other drugs were detected in this case. Two cases were concluded as having died from hanging. These had ethylphenidate concentrations of 1336 and 871 ng/mL. In these cases other drugs were present (ethanol, cocaine metabolites, sertraline, diphenhydramine, dothiepin and methiopropamine). The other 4 cases had ethylphenidate concentrations of 106, 136, 26 and 107 ng/mL and also included the presence of ethanol, methadone, zopiclone, sertraline, aripiprazole, 2-aminoindane, heroin metabolites, ketamine, cocaine metabolites, 5-APB/6-APB, diazepam and amphetamine.
Ethylphenidate toxicity is still an uncommon cause of death in the case mix of the Leicester forensic toxicology laboratory. There has only been a single case in which it was judged to be the primary cause of death, where the post mortem level was 2182 ng/mL. Comparison to the therapeutic levels of methylphenidate (up to 50ng/mL) and the previous published case report indicate that some of the concentrations detected in the other six cases may have led to toxic effects but there was either an alternative mechanism of death (such as hanging) or mixed drug toxicity. There were 2 cases in which ethanol was detected, but no methylphenidate, and no other markers of significant ethanol use, such as ethyl glucuronide, so although co-administration of cannot be fully excluded, it is unlikely.

WOP 14 DEATHS INVOLVING 'DOCTOR SHOPPING' AND THE MISUSE OF PRESCRIPTION DRUGS IN AUSTRALIA

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'Doctor shopping' describes individuals who present to multiple doctors or pharmacies to obtain a quantity of drugs beyond their therapeutic needs. The harms associated with non-medical use of pharmaceuticals are well documented, including dependence and addiction, toxicity and death. Doctor shopping has long been recognised in Australia as a serious public health issue that must be addressed to reduce morbidity and mortality associated with medication abuse, as well as the cost to Australians for inappropriately obtained medicines.
This retrospective, descriptive study aimed to examine a cohort of doctor shoppers in Australia to document the characteristics of the decedents, the drug most commonly involved, potential risk factors for negative outcomes in these cases, and to identify potential opportunities for death prevention.
The National Coronial Information System (NCIS) database was used to retrieve cases within Australia where one or more of the terms 'doctor shop'; 'prescription shop'; 'multiple doctor'; or 'multiple pharmacy' was identified in the coroner's finding, autopsy report, and/or police report, between January 2000 and December 2013. Only cases which were no longer under investigation by the coroner as at January 2015 were included. The data extract included demographic details of the deceased, the circumstances surrounding the death and the history of drug misuse from the police report and coroners' finding, as well as the post-mortem toxicology and autopsy results.
There were 264 cases involving doctor shopping (age range; median: 17-95; 39 years), of which 140 (53%) were males. The majority of deaths were unintentional (57.2%), followed by intentional self-harm (20.5%). Most occurred in the home (82.2%) and involved individuals who were unemployed (43.2%) or receiving government benefits (28%). The cause of death was drug-related in most cases (73.5%). The most commonly 'shopped' drugs were prescription opioids and benzodiazepines. Less than 5% of individuals had been identified by the national prescription shopping program prior to death; however some individuals had been banned by local medical clinics for drug-seeking. Mental illness was common in the cohort, reported in around half of the cohort (47%). Some individuals were drug-seeking at up to 22 different doctors prior to death. Recommendations were made by the coroner in 31 cases (11.7%), highlighting the need for a nation-wide prescription monitoring service.
This study revealed on average 19 deaths a year involving doctor shopping for pharmaceutical drugs. Many of these individuals suffered mental illness and very few had been identified as doctor shoppers prior to death. This study revealed opportunities for death prevention through earlier detection of problem drug users by doctors and pharmacists.

WOP 15 PAEDIATRIC DEATHS IN AUSTRALIA INVOLVING DIRECT DRUG TOXICITY 2003 - 2013

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Paediatric deaths pertaining to drug toxicity, both unintentional and intentional, comprise a small proportion of cases investigated by Australian Coroners each year. However, most are considered preventable and therefore represent an opportunity for injury reduction. Preliminary reviews of the medical literature indicate a scarcity of research surrounding paediatric drug deaths within Australia. This is the first in a series of epidemiological sub-studies of Australian drug toxicity deaths as part of the DEADSet (Deaths from Emergencies and Accidents Data Set) project.
The aim of this epidemiological study was to review and document all paediatric deaths (0 – 16 years old) in Australia over a 11 year period that were directly attributed to drug toxicity, in order to identify potential associated risk factors and prevention opportunities.

The National Coronial Information System (NCIS) is an internet based storage and retrieval system for Australian and New Zealand coronial cases. This was used to search and review all Australian deaths involving paediatrics aged 16 years and under, between January 2003 and December 2013, where the cause of death was directly attributed to drug toxicity and case investigation by the coroner was complete. Data extracted from the NCIS included police, toxicology and autopsy reports and Coroner's findings in conjunction with demographic data. The cases were examined and details documented; incident (location and circumstances), decedent (age, sex, intent, mental illness or co-morbidities, cause of death), toxicology findings (drugs detected, concentrations, specimen type, post mortem interval), and coroners recommendations.
There were 84 paediatric deaths in Australia directly related to drug toxicity between the years 2003 and 2013, the largest proportion of which were caused by a single drug (55 %). Fifty-two per cent of the cohort were male and aged between 13 to 16 years old (55 %). Most paediatrics died unintentionally (60%), at home (70 %) and, where documented, a quarter of all deaths had an existing mental illness or other co-morbidity. The most prevalent forms of paediatric death associated with direct drug toxicity were opioid toxicity (33 %), carbon monoxide poisoning (20 %) and volatile gas toxicity (11 %). Where opioids were detected, and prescribing history available, these were not prescribed to the decedent but diverted. Coroners recommendations were made in 11 % of cases, all of which were unintentional deaths with most referring to the involvement of drugs.
This study revealed 84 potentially preventable deaths of paediatrics attributed directly to drug toxicity. There were a number of potential risk factors identified, these being aged between 13 to 16 years old and with access to prescription opioids that were not intended for their use. The absence of information in some NCIS case files highlighted the need for a better collaborative approach in collecting and uploading Coronial data, in order to make case content more accessible and comprehensive.

WOP 16 REPORTING A DEATH INVOLVING OPIUM CONSUMPTION IN A LEGAL POPPY FIELD IN SPAIN

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Opium is a substance extracted from Papaver somniferum L. All parts of the plant exude a latex that contains morphine, codeine and thebaine but also non-analgesic alkaloids such as papaverine and noscapine. In Spain opium growing is allowed only after special permission for scientific or pharmaceutical purposes and harvest is supervised by the Spanish Health Ministry. Drug addicts often steal poppy capsules from the fields in the period from ceasing to flower until harvest to get free narcotics.
This work describes a sudden fatality involving opium consumption in a legal poppy field in Spain. The importance of toxicological findings and their quantitative toxicological significance along with autopsy findings, previous disease, and paraphernalia are discussed in order to clarify cause and manner of death.
A 32-year-old white caucasian male who was found unresponsive in a legal poppy field in the South of Spain. The emergency medical services responded to the scene where he was pronounced dead. Accordingly to friends at the scene the deceased had presented with about 30 min of convulsions; in spite of trying to keep his airway tract open they note that "he stayed airless". According to them the victim suffered from epilepsy. Body examination revealed rigor mortis compatible with the data of death. Internal examination showed micronodular hepatic cirrhosis, as well as visceral congestion. Tools found beside his body consisted of plain wood sticks with a blade razor, a fabric handle and paper.
A comprehensive toxicological screening for abuse and psychoactive drugs was performed in the deceased samples. This included ethanol and volatile analysis by headspace GC-FID in peripheral blood and urine, enzyme inmunossay in urine by CEDIA, and a basic drug screening in all samples (including paraphernalia) by GC-MS using modes full scan for screening/ confirmation and selected ion monitoring for quantitation. The peripheral blood, urine, vitreous, and gastric content contained the following concentrations of opiates expressed in mg/L (gastric content additionally also expressed in mg total): 0.10, 7.12, 0.23, and 14.80 (2.81 mg total) of thebaine, 0.13, 4.50, 0.13, and 6.60 (1.25 mg total) of morphine (free), 0.48, 0.88, 0.17, and 1.50 (0.28 mg total) of codeine. These tree opiates were also detected in the tools (paraphernalia) used by the deceased for opium consumption. Other toxicological findings were cocaine and cannabis metabolites.
This case describes a fatality associated with illicit poppy use. Apparently the victim stole poppy capsules and ingested an unknown quantity of the latex with the goal to obtain an euphoric effect. The cause of death was considered poly-drug toxicity with a preponderant role of thebaine and morphine. In addition, the epileptic condition of the deceased could have played a role. As far as we know, there are no previous reports of fatalities occurring in legal poppy fields.

THURSDAY, September 3rd

8.30-10.30 am SESSION 1 – In Vivo Forensic Toxicology

THOP 1 ACUTE INTOXICATION WITH CANNABIS IN A 12-MONTH-OLD GIRL: UNEXPECTED HIGH CONCENTRATIONS OF THC AND THC-COOH

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Cannabis is one of the most frequently used illicit drugs in the world and the incidence has been maintained more or less homogeneous over time. Because of the high consumption, paediatric intoxication by cannabis is increasingly common.
To describe a case of accidental cannabis poisoning in a 12-month-old girl, admitted to the Paediatric Emergencies, who presented with impaired consciousness (Glasgow 14), with no response to painful stimulus, paleness and cervical hypertonia. Preliminary toxicological analyses performed at the hospital gave positive results for cannabis and cocaine in urine. After a complaint of the hospital, the Judge asked for toxicological analyses. We received a blood sample obtained at the hospital.
Blood sample was subjected to a broad toxicological analysis. This includes screening analysis (for opiate, cocaine, cannabis, amphetamine, barbiturate and benzodiazepine compounds and for methadone and propoxyphene) by an immunoassay method (CEDIA) and confirmation and quantification by gas chromatography-NPD, liquid chromatography-DAD and gas chromatography-mass spectrometry (GCMS). In addition, and due to the fact that cannabis compounds require a specific extraction, the blood sample was submitted to a solid-phase extraction, after adding deuterium labeled internal standards. Heptafluorobutyric derivatives were obtained before analysis by GC-MS-EI.
Toxicological analyses gave positive results only for cannabis compounds. Unexpected high concentrations were found for both Đ9-tetrahydrocannabinol (THC) (217.5 ng/mL) and THC-carboxilic acid (THC-COOH) (280.7 ng/mL). In addition other cannabinoids were detected: cannabidiol (CBD) (8.45 ng/mL) and cannabinol (CBN) (17.26 ng/mL). Cocaine positive results obtained at the hospital, in the preliminary urine tests, were not confirmed in blood neither by CEDIA nor by GC-MS. Unfortunately, we did not receive urine to corroborate the results at the hospital. We do not have experience to interpret cannabis concentrations in blood of children. For this reason, we have compared the concentrations found in the girl with those obtained in the blood of 145 adults, 14 women and 131 men, with positive results for cannabis. The age ranged from 15 to 75 years old (median 35). THC concentrations ranged from 0.4 to 55.3 ng/mL (mean 8.2 ng/mL) and THC-COOH from 0.9 to 106.9 ng/mL (mean 15.9 ng/mL).
From the results obtained it was possible to confirm a severe acute intoxication with cannabis, probably hashish, just before going to the hospital. Since we did not receive urine, it was not possible to confirm the exposition to cocaine. Although toxic levels of cannabis in children are unknown, THC and THC-COOH concentrations found in the girl were the highest found in our laboratory.

THOP 2 PASSIVE INHALATION OF CANNABIS SMOKE IS DRUG ADMINISTRATION

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Passive exposure to cannabis smoke leads to inhalation of tetrahydrocannabinol (THC) by non-smokers. The extent, duration, and environmental conditions of exposure determine outcome on drug tests and behavioral changes.
The goal of this study was to evaluate the extent of THC exposure experienced by non-smokers and the resultant effects on drugs tests and behavior.
Three cannabis exposure sessions were conducted in which 6 smokers smoked cannabis cigarettes ad libitum for one hour in the presence of 6 drug-free, non-smokers. Sessions 1 and 2 were conducted with 5.3% and 11.3% THC cigarettes, respectively, in a non-ventilated room (3.2 X 4 X 2.1 m). Session 3 was conducted with 11.3% THC cigarettes in the same room with ventilation. Biological specimens (urine, oral fluid, hair) and behavioral measures from smokers and non-smokers were obtained at baseline and periodically after each session. Specimens were analyzed by GC/MS and LC/MS/MS for THC and carboxy-metabolite (THCCOOH). Area-under-the-curve (AUC) measures for drug concentration and behavior [Visual Analog Scale (VAS) measure of "Good Drug Effect"] for non-smokers were compared to those of smokers to determine the relative degree of THC intake.
THC and/or THCCOOH concentrations in specimens varied by individual, THC potency, and environmental conditions. The highest concentrations for non-smokers occurred after exposure in Session 2. Mean non-smoker AUCs (compared to smokers) from Session 2 were as follows: 11.2% (oral fluid); 5.9% (whole blood); 17.9% (urine, compared to amount of THCCOOH excreted in urine by smoking a single 3.55% cannabis cigarette); and 18.5% (VAS, good drug effect).
Extreme passive cannabis smoke exposure produced positive tests in biological specimens and drug-induced behavioral changes in non-smokers in a similar manner, but to a lesser extent, than in smokers. Consequently, passive exposure can be viewed as a form of drug administration.

THOP 3 LIFE-THREATENING AMLODIPINE POISONING: THE RESPECTIVE ROLE OF VENO-ARTERIAL ECMO, LIPID RESCUE AND MARS© BASED ON THE ANALYSIS OF PLASMA AMLODIPINE PHARMACOKINETICS

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Poisonings with calcium-channel blocker (CCB) represent the first cause of cardiotoxic-related fatalities. CCB poisoning management usually consists of supportive treatments and antidotes including catecholamines, calcium salts and euglycemic insulin but may require additional rescue therapies like lipid infusion (Intralipid® 20%), veno-arterial extracorporeal membrane oxygenation (ECMO) and toxin elimination enhancement based on hemodialysis or even MARS® (Molecular Adsorbents Recirculating System). Although veno-arterial ECMO seems promising in case of refractory cardiogenic shock, no treatment of drug-induced vasoplegia refractory to pharmacological agents was definitively proved as efficient.
We report two severely amlodipine-poisoned female patients (51 and 68 years-old) admitted to our intensive care unit after massive ingestions (3.6 and 0.9 g amlodipine, respectively), presenting refractory shock and treated with high doses of vasopressors, lipid emulsion, veno-arterial ECMO and MARS©. We discuss the modifications of amlodipine pharmacokinetics in relation to the various therapies used.
Measurement of plasma amlodipine concentrations was performed using a sensitive and specific assay using turbulent flow sample pretreatment prior to liquid chromatography coupled to tandem mass spectrometry. Briefly, 200 µL of amlodipine-D4 in acetonitrile were mixed to 200 µL of samples and centrifuged for 10 minutes at 14000 rpm. Thirty µL were injected in the LC-MS/MS system. Analytes were trapped onto a cyclone MAX column and eluted on a Hypersil Gold aQ column (Thermo, USA). Detection was achieved using a Quantum Ultra triple-quadrupole mass spectrometer with an electrospray ionization source in positive ion mode, using multiple reaction monitoring. The time-course of plasma amlodipine was modeled and the pharmacokinetic parameters calculated.
: The assay, fully validated according to European Medicines Agency's guidelines, was linear in a dynamic range of 2.5-200 ng/mL and suitable for drug monitoring and overdoses with amlodipine levels up to 1000 ng/mL after appropriate dilution. Amlodipine was successfully quantified in the plasma samples of both patients as well as in the different MARS© compartments including samples from the efferent and afferent blood, before/after albumin and charcoal cartridges, and in plasma treated after lipid emulsion administration, with the measurement of total and non-trapped fractions. Peak amlodipine concentrations were measured at 664 and 708 ng/mL (therapeutic range: 1-25), respectively. Due to the persistence of refractory vasoplegia despite supportive treatments, antidotes and ECMO, lipid emulsion (allowing decrease in free plasma amlodipine) and MARS© (allowing mild but significant amlodipine elimination by the different adsorption/exchange mechanisms: enrichment of the effluent solutions by ~18%) were used resulting in significant detoxification in both patients but only favorable outcome in the first patient significantly earlier treated after the ingestion. In the survivor, plasma amlodipine kinetics fitted a mono-compartmental non-linear model (terminal t1/2= 35.1 h; Vd/F= 13.5 L; Cl/F= 0.037 L/h).
In life-threatening amlodipine poisoning, refractory cardiovascular failure may benefit from the combination of ECMO, lipid rescue and MARS© allowing both pharmacodynamics support and significant decrease in plasma amlodipine concentrations. Future studies are still mandatory to better understand the mechanisms of action of these exceptional therapies to improve patient management.

THOP 4 USE OF BOTH THC-COOH FREE AND GLUCURONIDE TO ASSESS FREQUENCY OF CANNABIS CONSUMPTION

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Regular cannabis consumption is considered incompatible with safe driving. In Switzerland, drivers suspected of chronic cannabis consumption must undergo medical assessment of their long-term fitness to drive. A recently published placebo-controlled cannabis smoking study conducted at CURML demonstrated that a free 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH) whole blood concentration higher than 40 µg/L strongly suggests a heavy cannabis consumption, whereas a concentration lower than 3 µg/L is correlated with an occasional use. THC-COOH concentrations lying between these two thresholds do not allow for a clear conclusion. Therefore, an additional and/or better marker of frequency of cannabis use would be beneficial.
To study whether the whole blood levels of the glucuronide conjugate of THC-COOH can be used in addition to the established THC-COOH threshold for assessing the frequency of cannabis consumption.
23 heavy (≥ 10 joints/month) and 25 occasional smokers (≤ 1 joint/week) were enrolled in a clinical study conducted at CURML. Whole blood samples were collected before and up to 5 hours after inhalation. THC-COOH concentrations were determined by GC- or LC-MS/MS. For the present study, the blood samples were thawed after 3 to 6 years of storage at -80°C and were re-analyzed by a recently developed LC-MS/MS method which allows for rapid and simultaneous quantification of both free and glucuronidated THC-COOH. Receiver operating characteristics (ROC) curve analysis was performed to determine optimal cut-off values allowing the distinction between occasional and heavy smokers.
Concentrations of free THC-COOH measured in 2015 were on average 1.3 µg/L higher (5.3%) than the concentrations determined shortly after blood sampling. These results show that storage at -80°C ensures sample integrity. THC-COOH-glucuronide concentrations of occasional and heavy users ranged from < 5 to 51.5 µg/L and from < 5 to 358 µg/L, respectively. Occasional smokers showed molar concentrations of total THC-COOH (sum of free and glucuronide) between 0 and 0.168 µmol/L, which corresponds to 58 µg THC-COOH/L. For the heavy users, total THC-COOH concentrations ranged from 0.010 µmol/L (3.4 µg/L) to 1.095 µmol/L (377 µg/L). Based on the ROC-analysis

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two concentration thresholds could be established for discriminating heavy from occasional smokers with a specificity of 100%: 52 µg/L of THC-COOH-glucuronide (sensitivity of 40%) and/or 58 µg/L of total THC-COOH (sensitivity of 42%). For comparison, the previously established threshold of 40 µg/L of free THC-COOH was correlated with a sensitivity of only 20%.
Our results show that THC-COOH-glucuronide whole blood concentration is a useful parameter that complements free THC-COOH level to distinguish heavy from occasional cannabis users. The medical assessment of the fitness to drive could be improved if the concentrations of both free and glucuronidated THC-COOH are taken into account for classifying impaired drivers as heavy users. We suggest that a concentration higher than 60 µg/L of total THC-COOH (free + glucuronide) is indicative of regular use. This proposal is not only of interest in the Swiss context but could be also useful in other countries applying similar road safety policies.

THOP 5 WHEN ECSTASY TURNS OUT TO BE CLOZAPINE AT A DANCE EVENT...

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We report clozapine intoxication cases at a Belgian dance event with 30,000 attendees, most likely originating from clozapine tablets sold as party drugs. Case 1, a 20-year-old male, fainted in front of the medical station. He was dazed with normal pupils and reported taking 2-3 alcoholic drinks and half of a white 'ecstasy' tablet. He recovered after a few minutes. Case 2 was a 20-year-old male found comatose. He became agitated and 5 mg of diazepam was administered. A yellow pill was found in his pockets. At the emergency department, he had to be intubated after 3 episodes of obstructive breathing with desaturation and bradycardia. Eight hours after intubation, the patient was alert and left the hospital. Case 3, a 23-year-old female, was brought to the medical station due to restlessness and a fall on her head. She was agitated with dilated pupils, tachycardia and mild hypertension.
These cases are presented to make toxicologists and first aid professionals aware that the content of a pill consumed as party drug is as a rule uncertain.
All cases were part of a prospective toxicological study approved by the Ethics Committee and have undergone Systematic Toxicological Analysis (STA), which consists of screening by GC-MS and HPLC-PDA and targeted analyses (ethanol by headspace-GC-FID, GHB and "classic" drugs of abuse by GC-MS). Compounds identified by screening were quantified in blood using the most suitable in-house technique. Presence of clozapine was confirmed using a UHPLC-MS/MS method for common antipsychotics and metabolites.
In case 1, ethanol (0.7 g/l) and THC (1 ng/ml) was found. Screening with LC-PDA and GC-MS results were suggestive for clozapine. UHPLC-MS/MS analysis confirmed that clozapine (73.3 ng/ml) and norclozapine (25.5 ng/ml) were present. Case 2 had an ethanol level of 1.2 g/l and blood analysis was positive for diazepam, clozapine (243.5 ng/ml) and norclozapine (58.7 ng/ml). In case 3, ethanol (0.8 g/l), MDMA (635 ng/ml), MDA, lorazepam (32.6 ng/ml) but also clozapine (95.4ng/ml) and norclozapine (9.4 ng/ml) were found. The pill found on case 2 was identified as Leponex® 100 mg (clozapine). Clozapine is an antipsychotic used for treatment-resistant schizophrenia which can cause serious side effects like agranulocytosis. In clozapine-naïve subjects small doses of 25-350 mg can result in orthostatic hypotension, sedation, severe bradycardia/tachycardia, syncope and cardiac arrest. Serum clozapine concentrations are expected to be 350-600 ng/ml (trough concentration) for patients under chronic therapy (300-500 mg/day) with a clozapine:norclozapine ratio of 1.7-2 for smokers and 1.4-2.5 for non-smokers. In our cases, the calculated ratios are much higher (2.9, 4.2 and 10.1, respectively).
The clozapine blood levels in our patients, the elevated ratios and the clinical presentations argued against chronic clozapine treatment and were suggestive for recent intake and acute overdose. The clinical course of intoxicated patients may deviate from expected patterns and toxic agents may be missed by routine drug screening. Whenever there are life-threatening and/or unusual symptoms which are not in line with the information provided, the samples should undergo STA.

THOP 6 DETECTION OF 3-METHYLMETHCATHINONE, PAROXETINE, AND CLOTIAZEPAM IN HEAD HAIR AND/OR PUBIC HAIR SAMPLES BY LC/HR-MS ANALYSIS IN THREE DISTINCT FORENSIC TOXICOLOGY CASES

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Hair testing is considered to be one of the most efficient tool to investigate drug-related histories, particularly when the period of use needs to be tested back to many days or even months before sampling. Liquid chromatography/high-resolution mass spectrometry (LC-HRMS) represents nowadays one of the most specific and sensitive analytical techniques to detect psychoactive substances in hair samples following single or multiple drug exposures. Head hair and pubic hair LC/HR-MS analyses were employed to document exposure to psychoactive substances in three distinct forensic toxicology cases. In "case 1" pubic hair analysis was carried out in order to demonstrate even the personal use of some designer drugs by a drug dealer. In "case 2" head hair analysis was performed in specimens collected from a young woman about two months after she developed a relatively sudden change of mental status, neuromuscular hyperactivity and autonomic instability just after a disco night. In "case 3" segmental head hair and pubic hair analyses were carried out in the context of a drug-facilitated crime investigation, about one month and a half after the overnight robbery in an old man shop. Illicit and pharmaceutical drugs were searched in hair samples from the above cases by means of LC-HRMS with an Orbitrap® analyzer.

Head hair segments and pubic hair were decontamined and pulverized with a ball mill, and, after the addition of internal standards, extracted with methanol: trifluoroacetic acid 9:1 at 45 °C for one night, or by SPE using Bond Elute Certify columns. The obtained extracts were analyzed on a Thermo Scientific Accela 1250 UHPLC system equipped with a Hypersil Gold PFP analytical column (2.1 x 50 mm, 1.9 µm particle size), coupled to a Thermo Scientific single-stage Exactive HCD MS system.
3-methylmethcathinone (3-MMC) was found to be present (25.8 ng/mg) in "case 1" pubic hair sample, whereas several other searched designer drugs were found to be absent. 3-methylephedrine and 3-methylnorephedrine, metabolites of 3-MMC, were identified in the same sample, thereby proving the 3-MMC intake by the drug dealer. In "case 2" the head hair proximal (0 – 1.5 cm) segment showed the presence of the pharmaceutical drug paroxetine (2.1 ng/mg), while the second (1.5 – 3 cm) and distal (4.5 - 6 cm) segments were both negative. The finding was consistent with the woman clinical status after the disco night, which looked like a serotonin syndrome. In "case 3" the analysis of several 0.5 cm head hair segments and pubic hair revealed the presence of the benzodiazepine clotiazepam in the pg/mg range. The old man was clearly exposed to the drug but an accurate evaluation of results was necessary in order to provide police with information about possible over-exposure during robbery.
Segmental head hair and pubic hair LC-HRMS analysis results allowed to document the exposure to illicit or pharmaceutical drugs in three distinct forensic toxicology cases.

THOP 7 A PROSPECTIVE PILOT STUDY OF MEASUREMENT OF BLOOD CONCENTRATIONS OF ROUTINE MEDICATIONS IN PATIENTS TREATED IN THE INTENSIVE CARE UNIT

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Patients in the intensive care unit (ICU) are given a variety of analgesic and sedative medications. The amount of drugs that the patients receive is based on standard dosages primarily derived from clinical studies with a limited number of patients included. Most of the patients treated in the ICU have a varying degree of multiple organ failure, which affect the drug metabolism. Possibly, the drug concentrations achieved with the standard dosages administered can vary significantly between patients. Intoxications due to suicidal purposes may result in intensive care for those who survive. Upon arrival to the ICU it is often unknown what type of medication or drugs these patients have taken and what blood concentration it may result in. The central ICU at Uppsala University Hospital has together with the Swedish National Board of Forensic Medicine initiated a project where blood concentrations of routine medications will be studied in relation to the amount given. The measured doses and concentrations will be related to the patient's level of heart-, liver- or kidney failure and to the clinical response by measuring the patient's sedation level and assessed pain. Antemortem and postmortem blood drug concentrations will be compared in patients that die in the ICU. It will also be studied whether self-intoxicated patients have taken drugs that were primarily not suspected.
The aims of this presentation are to describe the project concept and to present the results from a pilot study with special focus on logistics and the two substances propofol and fentanyl.
A prospective, observational pilot study of ICU patients was performed during one month. Blood samples were collected according to routine procedures upon the patient's arrival at the ICU, and then twice daily until discharged. The samples were screened for medications and drugs of abuse using UHPLC-TOF. The analysis of propofol was done by GC-MS and the limit of quantitation (LOQ) was 0.05 µg/g. For fentanyl, LC-MS/MS was used with an LOQ of 0.05 ng/g.
Sixty-nine patients were included which resulted in 370 blood samples available for drug analysis. Propofol and fentanyl were the most requested substances followed by clonidine, paracetamol (acetaminophen), morphine, dexmedetomidine, midazolam, ketobemidone, ketamine and thiopental. The mean, median and range of propofol concentrations in 222 samples were 1.08 µg/g, 0.62 µg/g and 0.06-5.6 µg/g, respectively. The mean, median and range of fentanyl concentrations in 173 samples were 1.26 ng/g, 0.85 ng/g and 0.05-6.2 ng/g, respectively. During the study period, four intoxications were treated at the ICU and all survived.
The variety of drug concentrations observed in relation to standard dosages need to be further investigated in a larger ICU population. Possibly, this project can result in more knowledge about concentrations and effects of analgesic and sedative medications, and how these are metabolized in the body of critically ill patients. In the long run, information that might fill in knowledge gaps regarding drug concentrations after death will be obtained.

THOP 8 EFFECTS OF RECENTLY-EMERGED SYNTHETIC CANNABINOIDS ON LOCOMOTOR ACTIVITY IN MICE

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The consumption of synthetic cannabinoids as new psychoactive substances (NPSs) has become widespread despite regulatory control measures. Our continuous survey of NPS in the Japanese market has revealed the emergence of new types of synthetic cannabinoids in illegal products [1-3]. However, little information is available for their pharmacology. We previously reported that six newly-emerged synthetic cannabinoids suppressed the locomotor activity in mice [4]. In this study, we examined the effects of 44 recently-emerged synthetic cannabinoids on locomotor activity in mice and compared with those of JWH-018, a representative synthetic cannabinoid, to evaluate their pharmacological activity in vivo. Each tested compound was dissolved in a vehicle consisting of 5% DMSO, 5% Emulphor® EL-620 and 90% saline. This vehicle alone served as the control treatment. The vehicle and all compounds were intraperitoneally (i.p.) administered. The locomotor activity of mice (C57BL/6j male, 9 weeks old, n=3 or 4) was detected with a passive infrared sensor and recorded for two days. On the first day: vehicle-treatment, the second day: drug-treatment (5 mg/kg i.p. at beginning of dark period (19:00-7:00)). The total amounts of locomotor activity were calculated as percentages of control values obtained from the vehicle treatment. Statistical analysis was determined by Student's t-test and a P value < 0.05 was considered to be significant.
Among 44 synthetic cannabinoids, 31 compounds significantly decreased the total amounts of the locomotor activityby 41-99% compared to the base line, and one compound significantly increased the activity during 6-hour period after injection. Additionally, 12 compounds showed no significant effect even at a dose of 5 mg/kg. Especially, (1) the six indazole-carboxamide-carboxylester derivatives (MA-CHMINACA, MDMB-CHMINACA, AMB, 5-Fluoro-AMB, 5-Fluoro-ADB and FUB-AMB) and an indole-carboxylester derivative: NM-2201 significantly suppressed the locomotor activity to less than 10% and stronger than JWH-018 (indole-carbonyl derivative). In addition, these seven compounds produced some symptoms such as seizures, straub tail and immobility in mice after a few minutes of the injection. (2) A 1H-indazole derivative: AB-CHMINACA significantly reduced the locomotor activity. However, its 2H-indazole isomer: AB-CHMINACA 2H-indazole analog significantly increased the locomotor activity. (3) Although indole-carboxylester derivatives: NM-2201 and 5-Fluoro-QUPIC (5-Fluoro-PB-22) significantly decreased the locomotor activity, their indazole analogs: 5-Fluoro-SDB-005 and 5-Fluoro-NPB-22 had no significant effect.
Among 44 recently-emerged synthetic cannabinoids, 31 compounds significantly decreased the locomotor activity in mice. In addition, the seven compounds of them suppressed the locomotor activity stronger than JWH-018. [1-3] Uchiyama N., et al., Forensic Toxicol. online available, DOI: 10.1007/s11419-015-0268-7 (2015), Forensic Toxicol. (2014) 32, 266–281, Forensic Toxicol. (2014) 32, 105–117. [4] Uchiyama N., et al., TIAFT2014, abstract book pp52.

THOP 9 DRUG SCREENING AND ILLICIT DRUG USE IN A TWO-SITE SOLID ORGAN TRANSPLANT PRACTICE

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Solid organ transplantation is an increasingly common therapeutic intervention for a variety of end-stage diseases. Substance abuse has serious implications in the transplant population, both in terms of selection to receive a grafted organ, and in post-transplantation outcomes such as adherence to prescribed medications. However, most studies of substance abuse in solid organ transplantation have been focused on alcohol or tobacco, rather than illicit drugs. In addition, the bulk of the literature focuses on liver transplantation, with much less known about substance abuse in cardiac, renal, or other transplant populations. To address these gaps in the literature, this study describes illicit drug use in transplant patients at two sister sites, Mayo Clinic Arizona (MCA) and Mayo Clinic Rochester (MCR), as a foundation for future prospective studies. This study was intended to characterize the prevalence of illicit drug use in a two-center solid organ transplantation practice. All urine drug screens and associated confirmation results performed between 1 Jan – 31 Dec 2014 for MCA and MCR patients were retrieved from the laboratory information system. Pediatric results were removed from the study. Medical records were then reviewed to distinguish solid organ transplant patients from the general population, and gather demographic information relevant to the study, including transplant date, prescribed medications, etc.
Over 1150 screening and confirmation results for almost 900 unique patients across both sites were reviewed. Urine drug screens are ordered routinely with all pre-transplant evaluations for liver (MCA and MCR) and cardiac (MCA only) transplant practices. Screening in other settings (e.g., post-transplant evaluations) is typically done for suspicion of substance abuse. Urine drug screening in the kidney transplant population is less uniform due to a large subset of anuric patients. Illicit drug use was limited to cocaine, marijuana (THC), and methamphetamine in this population; data regarding misuse of potentially prescribed drugs (e.g., opioids) was difficult to reliably extract from the medical record. Unsurprisingly, THC was the most common illicit drug at both sites, confirmed positive in nearly 10% of patients. Confirmed use of THC was over two times as common in MCA liver transplant candidates than in the corresponding MCA cardiac candidate pool (9.5% vs 4.6%, respectively). Cocaine use was confirmed in 1 MCR patient and 3 MCA patients. MCR also had 2 confirmed methamphetamine positive patients whereas MCA did not report this compound, which may reflect geographic differences in drug use. 3 additional MCR patients had amphetamine positivity attributed to prescriptions (Adderall or Vyvanse); interestingly, all 3 of these patients were also THC positive. This descriptive study adds to the literature regarding illicit drug use in solid organ transplant populations. MCA and MCR are two of the most prolific transplant centers in the United States; characterization of this population serves as the foundation for future prospective studies regarding substance abuse in solid organ transplant.

11.00-1.15 pm SESSION 2 – Hair Analysis

THOP 10 HYDROMORPHONE IN HAIR: AN INDICATOR OF HEROIN INTAKE VS CONTAMINATION?

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The differentiation of external hair contamination by drug residues from actual drug use is still a challenge in the interpretation of hair analysis results. In particular, when drug dealers deny dealing and claim possessing drugs solely for personal use. Heroin is one of the drugs for which contamination can occur. Checking for presence of metabolites may help to distinguish between contamination and actual intake. Heroin is metabolized to 6-mono¬acetylmorphine and morphine. Morphine is further metabolized to a minor extent to hydro¬morphone. However, hydromorphone is also used as a pharmaceutical in pain medication and may also be present as a metabolite of hydrocodone.
The present study investigates the applicability of the metabolic ratio of hydromorphone to morphine as indicator to distinguish between intake of heroin and external contamination in cases where hydromorphone or hydrocodone intake can be excluded.
Hair samples were collected from patients with opioid medication, heroin addicts, individuals enrolled in an opioid substitution therapy, and heroin dealers. Inclusion criteria for the cohort was a morphine concentration above the Society of Hair Testing (SoHT) cut-off of 200 pg/mg (n=211). All samples were not only analyzed for morphine, 6-monoacetylmorphine, and codeine, but also for acetylcodeine, dihydrocodeine, hydromorphone, hydrocodone, oxymorphone, and oxycodone. Hair was washed and extracted in two steps (methanol and methanol/hydrochloric acid 0.1 N). Extracts were analyzed by a validated LC-MS/MS method approved in our accredited routine. A controlled external contamination study using heroin was performed with eight volunteers. Additionally, 20 heroin samples seized in the Canton of Zurich (Switzerland) were analyzed for the primary presence of other opioids including hydromorphone. Individuals (n=211) were divided in two groups: substitution group (morphine, medicinal heroin, morphine with additional heroin use; n=84) and forensic group (heroin dealers/users; n=125) based on case information and hair analysis results; two cases were excluded due to hydromorphone intake. Hydromorphone was detected in all hair samples except for 30 % of the samples in the forensic group. Hair samples from the substitution group displayed significantly (Mann-Whitney, $p < 0.0001$) higher metabolic ratios of hydromorphone to morphine (0.016 to 0.29, median: 0.092) than those from the forensic group (0 to 0.25, median: 0.023). All seized heroin street samples and hair samples from the controlled contamination study with heroin were negative for hydromorphone. These investigations are indicating that hydromorphone is solely formed after body passage.
The hydromorphone to morphine ratios could be used to distinguish morphine/heroin use from external contamination in hair testing if hydromorphone and/or hydrocodone use can be excluded. When analyzing hair samples for opioids, testing for hydromorphone, hydrocodone, dihydrocodeine, oxymorphone and oxycodone is highly recommended.

THOP 11 UTILITY AND ALTERNATIVES OF COSMETICALLY TREATED HAIR FOR THE SENSITIVE DETECTION OF DRUGS AND ALCOHOL

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The majority of the published studies concerning the influence of cosmetic treatment on drugs and EtG in hair involve few, statistically insignificant number of samples and are in-vitro studies, often not representative of authentic hair samples.
To assess the utility of cosmetically treated authentic hair samples for the detection of drugs and alcohol in a large statistically significant population.
The positivity rate, the 1st, 5th, 50th, 95th and 99th percentiles of five amphetamines, cannabinoids, cocaine, four opiates, methadone, buprenorphine, seven benzodiazepines and ethyl glucuronide (EtG) in 9488 non-treated and 1026 cosmetically treated (900 coloured and 126 bleached) authentic hair samples was compared. Moreover the positivity rate of the above drugs in 33262 authentic urine samples tested for the same context was also compared to that in cosmetically treated hair samples. Analytical methods used were accredited for forensic purposes at the cut-offs defined by the German driving licence re-granting medical and psychological assessment (MPA) guidelines.
Considering only the drug classes for which at least 10 positive samples were detected, the positivity rate in non-treated hair was highest for alcohol (4.50%; measured using EtG at concentrations ≥ 7 pg/mg hair), followed by THC (2.00%), cocaine (1.75%) and amphetamine (0.59%). While the 1st to 99th percentile range was significantly lower for drugs in cosmetically treated hair, compared to non-treated hair, no significant change was observed for EtG. Additionally, no significant difference in the positivity rate was observed between cosmetically treated hair and non-treated hair for both drugs and EtG at both the 95% and 99% significance level.
Although this study needs further investigation, it is the first attempt to study the influence of cosmetic treatment on the positivity rate in hair for both drugs and EtG. Furthermore, this study is the first to indicate that cosmetically treated hair is not necessarily useless to detect drugs and/or alcohol consumption. Moreover, particularly for alcohol abstinence monitoring, as for the MPA in Germany, it seems that EtG in hair presents a much better alternative than urine testing, even when cosmetically treated hair is analyzed. Hair analysis is a powerful tool, often the only means to detect retrospective drugs and/or alcohol consumption even in dyed or bleached hair.

THOP 12 ESTABLISHMENT OF A DIAGNOSTIC PROBE FOR THE DISCRIMINATION OF EXOGENOUS GHB IN HUMAN HAIR

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Many voluntary intoxication cases remain unresolved by investigators, particularly those related to the GHB drug that show a short window of detection in blood (6 hrs.) and urine (10-12 hrs.). In order to increase the detection time, few analytical methods are now proposed for the analysis of GHB in human hair. However, the basal level of GHB measured in hair segments show a significant variability. Therefore, a reliable and sensitive methodology is required to precisely define the cut-off concentrations associated with an exogenous ingestion of GHB.

The aim of this study is to present a new tool of investigation that could be used for the detection and quantification of GHB in human hair by Liquid Chromatography (ESI) tandem Mass Spectrometry (LC-MS/MS). The proposed methodology is based on the application of a diagnostic probe that enables a normalization of signals in the different hair segments. Specifically, the probe consists of a concentrations ratio expressed as GHB / precursor (GABA), or GHB / metabolite (succinic acid).

Following official guidelines from the Society of Hair Testing, a new protocol has been successfully validated for the selective extraction of GHB (4-hydroxybutanoic acid), GABA (4-aminobutanoic acid) and succinic acid from human hair. In this study, different HILIC chromatographic columns (Primesep-N, Obelisc-N, Inertsil amide) were tested for adequate separation of all targeted substances. The separation of positional isomers (i.e. GABA, 3-aminobutanoic acid, 2-aminobutanoic acid / GHB, 3-hydroxybutanoic acid / succinic acid, methyl malonic acid) that can cause possible isobaric interferences was optimized in laboratory. Finally, the extraction of all substances was achieved using state of the art of purification techniques such as mixed-mode polymeric solid-phase extraction (SPE) cartridges, and liquid-liquid extraction (LLE). Both techniques were compared during the validation process. A GHB-d6 solution was also used as an internal standard.

Satisfactory extraction recoveries were obtained from 25 mg hair samples processed under alkaline conditions during 40 minutes at 75°C. The sensitive method was proved to be linear ($R^2 > 0.99$) with LOD values surrounding 0.15 ng/mg. The diagnostic probe was tested on normal hair specimens to determine the basal physiological level of GHB. After validation, the probe was applied to positive hair specimens collected from one Canadian victim involved in a drug-facilitated crime (DFC) case. The presence of GHB was confirmed by LC High Resolution Mass Spectrometry (HRMS).

A sensitive diagnostic probe has been validated for the discrimination and quantification of exogenous GHB in human hair using a LC-MS/MS method. To our best knowledge, this innovative methodology used to normalize the levels of GHB in hair segments has not yet been reported in the literature.

THOP 13 MASS SPECTROMETRY IMAGING FOR DEPICTING DRUG INCORPORATION INTO HAIR

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Hair is often compared to a tape recorder for drug use history. However, the mechanisms of drug incorporation into hair are still under discussion among forensic toxicologists.

This study was designed to depict the incorporation of ingested drug into scalp hair by mass spectrometry imaging (MSI). MALDI–TOF MSI was performed on the longitudinal sections of single scalp hair shafts plucked/cut from volunteers who took methoxyphenamine (MOP), a nonregulated analogue of methamphetamine. In addition, conventional sectional analysis by LC-MS/MS was performed for each 1 mm segment of single hair specimens to support quantitative aspects of MSI results. Based on these results, forensically crucial issues, including the site of drug incorporation into hair and time resolution in estimating drug use history, will be presented.

Specimens: Hair specimens (straight, black) were collected from volunteers (e.g., by plucking out with the roots intact, 1, 2, 3, 7, 14 days after a single oral administration of an OTC medicine containing 50 mg MOP hydrochloride). All protocols were approved by the ethics committee of Osaka Medical College. MSI: The lengthwise cutting of hair shafts was achieved by freeze-sectioning using a specially customized microtome to detect the drug inside the hair shaft. MALDI-TOF MSI was performed on ultrafleXtreme (Bruker Daltonics) in the TOF/TOF mode, after deposition of a CHCA-based matrix solution. Segmental analysis: A single hair specimen was divided into ten 1.0-mm segments from the root-side end, and each segment was pulverized and extracted with 50 mM acetate buffer in a disposable 2-mL safe-lock tube, followed by extraction with chloroform-isopropanol (3:1). LC-MS/MS was carried out on a Shimadzu Prominence LC system linked to an AB Sciex QTRAP 5500 hybrid triple quadrupole linear ion-trap mass spectrometer (ESI positive, SRM mode).

A substantial concentration of MOP was observed first in the hair bulbs after ingestion, while only a small portion appeared to be incorporated into the hair matrix, forming a 2–3 mm distinctive drug band with tailing. Comparable amount of the drug was also incorporated into the keratinized hair shaft in the upper dermis zone, forming another distinct band of about 2 mm. These two bands appeared to be connected with insignificant MOP-positive area forming an approximate 6–7 mm two-peak MOP-positive area, which moved toward the distal side thereafter, likely following the strand's growth rate. No notable influence of sample preparation on the MSI results was observed. To examine the chronological resolution of hair analysis, we also analyzed single hair specimens from volunteers who took MOP at various time intervals. The MOP-positive areas barely separated into each administration at 11-day interval.

This study provides direct and visual evidence that there are two record heads to the tape recorder, which unfortunately cause overlap of the recordings and deteriorate its chronological resolution down to about eleven days or perhaps longer. These facts should be taken into account in interpreting the result of any hair analysis.

THOP 14 LONGITUDINAL SCANNING OF DRUGS OF ABUSE IN HAIR USING DIRECT ANALYSIS IN REAL TIME (DART) HIGH RESOLUTION MASS SPECTROMETRY

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Current forensic hair analysis methods comprise decontamination, extraction and/or digestion, clean-up and subsequent analysis by liquid- or gas chromatography (tandem) mass spectrometry (MS/MS). When a retrospective timeline of drug intake is needed, typically 1-3 cm long hair segments are being analyzed following similar methodologies, thus yielding a very laborious and time-consuming workflow that offers only a rough estimate due to the average hair growth rate of approximately 1 cm/month. Recently, hair imaging methods using matrixÐassisted laser desorption ionization mass spectrometry (MALDI-MS) were reported, but these methods focus on individual hairs, leading possibly to misinterpretations due to the different hair growth phases of individual hairs. Ambient ionization of intact locks of hair would solve those issues.
The aim of the present study was to explore Direct Analysis in Real Time (DART) ambient ionization coupled to different mass analyzers, for rapid longitudinal profiling of drugs of abuse in hair.
The DART helium gas temperature and the accuracy of the probed hair zone were optimized for the analysis of delta-9-tetrahydrocannabinol (THC) and cocaine. After developing and testing the method on spiked hair samples, drug user hair samples were scanned. The performance of different mass analyzers (linear ion trap; orbitrap; quadrupole-orbitrap; triple quadrupole; quadrupole-traveling wave ion mobility-time-of-flight) was critically compared. With the DART hair scan and an orbitrap MS instrument, THC and cocaine could be detected in hair samples from different drug users. The longitudinal concentration profiles obtained, clearly suggested that the DART method can be used for retrospective timeline assessments. Coupling of the DART ionization source to more advanced MS instruments enhanced the method sensitivity and selectivity: for example, the use of a quadrupole-orbitrap mass analyzer resulted in a 10-30 fold increase in sensitivity, in accordance with expectations. The highest sensitivity was reached when combining the DART hair scan with a triple quadrupole instrument operated in SRM-mode. Of course, in the latter case only targeted analysis can be done, while the other mass analyzers studied yielded full scan data, enabling retrospective data analysis for other drugs, e.g., new designer drugs. Apart from the critical comparison of the different DART-hair scan set-ups, the ability of DART-scanning for retrospective assessment of time of drug intake will be discussed versus conventional segmented hair analysis techniques. Next to this, a critical assessment will be made of the DART-MS method versus the sensitivity recommendations of the Society of Hair Testing.
A simple hair testing method has been developed, which provides longitudinal profiles of drugs and a better retrospective estimate of time of drug intake versus conventional 1-3 cm segmented hair analysis. The method combined with full-scan high resolution accurate (tandem) mass spectrometry can be used as a rapid untargeted pre-screening tool.

THOP 15 OCCUPATIONAL EXPOSURE TO KETAMINE DETECTED BY HAIR ANALYSIS: A RETROSPECTIVE AND PROSPECTIVE STUDY

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Ketamine (KT) is mainly being used to induce and maintain general anaesthesia in combination with sedative drugs in human and animals. Because of its dissociative and hallucinogenic effects, KT has become a recreational drug in a variety of social settings as well as in drug-facilitated sexual assault and may be included in the panel of drugs of abuse that are controlled in driving under the influence (DUI) ascertainment. In the local driving licence re-granting protocol, nearly 2500 subjects/year undergo legal medical ascertainment and are routinely tested in hair for stupefying and psychotropic substances including KT. About 2 % of yearly samples are positive for KT at a 0,05 ng/mg cut-off and its metabolite nor-ketamine (NK). In subjects positive for KT and NK in hair, a possible "contamination" from professional exposure was invoked by a veterinary physician, formerly convicted for DUI of alcohol but rejecting any contact with drugs of abuse. The man was using KT as an anaesthetic in his daily surgery occupation. A study to verify the possibility of professional exposure to KT in a veterinary setting was thus envisaged.
Man (7) and female (4) veterinary physicians were recruited on a voluntary base. Detailed information was collected on their habits, use of drugs, professional practice, frequency and mode of using KT in their activities, use of hair treatment. Head hair and pubic hair were collected. Hands and skin were examined. Two naïve subjects starting their professional practice at a local veterinary clinic, were recruited and their hair were collected before and after a month of "normal" clinic activity in a blind trial. Hair samples were cut according to their length, washed, pulverized and 25 mg were extracted and analyzed by liquid chromatography coupled to high accuracy, high resolution mass spectrometry in an Orbitrap, with a limit of quantification of 0,01 ng/mg.
All the examined head and pubic hair turned to be positive for KT, at a concentration varying from 0,03 to 0,10 ng/mg. KT was present in 80% of cases, from 0,01 to 0,04 ng/mg. Washing residues were always negative. For the two naïve subjects, hair and beard were negative before they started their activity, and positive one month later. A mechanism for a systemic incorporation of KT from skin into the body was proposed. Comparison with a population of subjects undergoing driving licence re-granting evidenced a higher range of concentration in this latter setting (0,05 – 10 ng/mg for KT, absent to 0,1 ng/mg for NK).
The applied experimental protocol evidenced the possibility of incorporating the anaesthetic agent KT when exposed in a clinical veterinary setting. The possibility of unaware exposure to KT is demonstrated, even though the concentration levels in hair were lower in the veterinary setting than in subjects formerly convicted of DUI. Possible adverse systemic effects by unaware KT exposure need to be studied and concerns have arose as to healthy and safe workplace policies.

THURSDAY, September 3rd

THOP 16 WHEN KISSING CAN RESULT IN AN ADVERSE ANALYTICAL FINDING DURING DOPING CONTROL: ABOUT 2 CASES WHERE HAIR TESTING WAS DETERMINANT FOR THE ATHLETE

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In doping, the sanction is ineligibility for a specific period. Although the code of WADA considers a doping offence when a banned drug is found in the athlete's urine, irrespective of reasons, this is not the case in forensic medicine, where additional investigations can be performed, based on scientific literature evidence.
Sometimes, the sport authorities have accepted that adverse analytical findings can be documented by alternative methods, such as hair testing. The aim of this presentation is to review 2 recent cases involving cocaine and the perspectives of hair testing in doping control.
Hair samples were collected and analysed for cocaine and metabolites by GC/MS using an accredited method. LOD of the procedure is 10 pg/mg.
In urine, athlete A tested positive for benzoylecgonine at 151 ng/mL. Athlete B tested positive for benzoylecgonine at 60 ng/mL. It is considered that the exposure occurred about 12 and 30 hours before urine collection, respectively This is based on the delay between contact with the girls and urine collection. Given the properties of cocaine based on published controlled studies, it was submitted and accepted by the anti-doing panels, including the Court of Arbitration for Sport that the total amount of cocaine that entered into the athletes' body was less than 5 mg. Segmental analyses of hair, collected 4-6 weeks after the offense were negative for both athletes. There is scientific literature indicating that the minimal detectable dosage of cocaine in hair is about 15 mg. Thus, the hair findings confirmed the urine interpretation. A review by the lawyers of the potential sources of contamination proposed that it was more than likely that the origin of cocaine was a consequence of kissing a girl who had recently sniffed the drug with low amounts still on her lips. The anti-doping panel accepted these evidences and judged that no fault or negligence has to be retained. Instead of the standard ineligibility of 2 years, one athlete was sentenced for 7 weeks and the other was allowed to return to playing with immediate effect.
It is the opinion of the author that hair testing will present a more accurate reflexion about doping practices than some urine tests. There is an increase in papers dealing with hair applications in doping, and a global approach about the interest of alternative specimens (including oral fluid to document recent cannabis use) should be developed among active researchers. As there is a debate about the true prevalence of doping (some national bodies consider that the prevalence is far higher that the official 1-2 % of AAF), it seems that it is the responsibility of WADA to consider hair as a valid specimen.

THOP 17 DETERMINATION OF HYDROXY METABOLITES OF COCAINE IN HAIR SAMPLES FOR PROOF OF DEFINITE CONSUMPTION

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Hair testing is a common technique used for the determination of cocaine consumption. The target analytes for testing of Cocaine consumption, according to the Society of Hair Testing (SoHT) guidelines, are Cocaine and its metabolites Benzoylecgonine, Ecgoninemethylester, Cocaethylene and Norcocaine. After consumption, cocaine and its metabolites are incorporated into the hair through blood, sebum and sweat. When cocaine is used, usually sniffed as a powdery substance, it is possible, that hair can be contaminated with cocaine powder externally. It is also known that metabolites, which are formed in the body, such as Benzoylecgonine, can also be present in Cocaine powder as a degradation product formed through hydrolysis. Norcocaine and Cocaethylene are described as possible processing impurities in street Cocaine samples. Because of this, we were looking for minor metabolites of cocaine in hair samples, which are only formed during body passage, to prove cocaine consumption without doubt.
Testing for hydroxy-metabolites of Cocaine, formed via body passage, could be an alternative to prove Cocaine consumption. The known hydroxy-metabolites, which are formed in the liver, are para-/ meta- hydroxycocaine, para-/ meta- Benzoylecgonine. Therefore, the aim of the study is to show the applicability of LC-MSn to detect Cocaine-hydroxy-metabolites in hair samples.
The first approach to detect these metabolites was performed by LC-QTOF-MS (ABSciex 5600) from hair extracts, which tested positive for cocaine and Benzoylecgonine in high concentration ranges. All selected samples tested positive for the hydroxy-metabolites mentioned above. The acceptance criteria for detection were the accurate mass and the MS/MS fragmentation. Out of this approach, a LC-MS/MS method in MRM mode on an ABSciex 6500 QTrap-MS was generated, to reach higher sensitivity. After two washing steps to remove external contamination and cutting, the hair specimens were extracted with methanol and analyzed with a LC-MS-system. Two MRMs (target and qualifier) were recorded for each analyte.
The results of the MRM- measurements revealed positive results with two peaks for each of the hydroxy metabolites of Cocaine, Benzoylecgonine and Norcocaine, which can be explained with the hydroxylation in meta- and para-position. 37 hair samples, which had been positive for Cocaine (0.05 - 50 ng/mg) were tested for the presence of various Cocaine-metabolites with the new MRM method. It was possible to detect Benzoylecgonine (>0,05 ng/mg) in 32 cases (86%), Norcocaine (>0,05 ng/mg) in 18 cases (48%) and at least one of the hydroxy-metabolites in 34 cases (91%).
Testing for hydroxy-metabolites of Cocaine, Benzoylecgonine and Norcocaine could deliver valuable additional information to distinguish between consumption and external contamination. Further investigations are necessary.

THOP 18 INFLUENCE OF THERMAL HAIR STRAIGHTENING ON COCAINE IN HAIR

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It has been shown that cosmetic treatment like bleaching and perming may lead to an important decrease of cocaine content in hair. Currently, hair straightening has become a daily hair treatment especially for women. However, no studies exist about the influence of this heat treatment on cocaine content in hair.
The aim of this preliminary study was to investigate the effect of thermal hair straightening on cocaine, benzoylecgonine (BZE) and cocaethylene concentrations in hair.
7 positive cocaine positive hair samples were treated in vitro with a hair straightener: hair was put 30 times in contact with heated iron plates (200°C) during 2 s corresponding to a total time of contact of 1 min. After washing, pulverization, incubation in phosphate buffer at pH 6 during 2 h in an ultrasonic bath, solid-phase extraction and derivatisation with pentafluoropropionic anhydride and pentafluoropropanol, analyses were performed by GC/MS in electron impact mode. Cocaine, BZE and, if determined, cocaethylene concentrations in straightened hair strands were then compared with those in the corresponding untreated strands.
In all seven hair samples the concentration of cocaine decreased after treatment ranging from 44.6 % to a total loss (average 78.8 %). In contrast, higher concentrations of BZE were determined in hair with an increase ranging from 3.3 to 48.2 % (average 20.5%). In 4 of 7 samples cocaethylene was additionally analysed, resulting also in a decrease ranging from 17.6 to a total loss (average 55.4 %).
The decrease of cocaine and cocaethylene content may be explained by thermal destruction of the substance. The increase of BZE may be due to chemical transformation of cocaine into BZE. This preliminary study indicates that thermal hair straightening leads to an important decrease of cocaine concentration in hair and significantly changes the ratio between cocaine and BZE concentrations. Therefore, this frequent hair treatment should be considered for a correct interpretation of hair results. However, these results should be confirmed by in vivo studies over several weeks in order to consider further influence as washing out effects from damaged hair.

THURSDAY, September 3rd

2.45-4.15 pm SESSION 3 – New Technologies I

THOP 19 IDENTIFICATION OF NEW PSYCHOACTIVE SUBSTANCES AND THEIR METABOLITES USING HIGH RESOLUTION MASS SPECTROMETRY FOLLOWING A NOVEL STRUCTURED WORKFLOW

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Liquid chromatography (LC) coupled to a quadrupole time-of-flight mass spectrometer (QTOF-MS) instrument provides sensitive, full-spectrum MS data with high mass accuracy for identification of both known and unknown compounds. There are currently around 450 new psychoactive substances (NPS) on the market. Presently, NPS are a growing problem in many communities, responsible for an increasing number of severe or even fatal intoxications. Some of them have no commercially available reference standards making it challenging to develop targeted methods for their analysis. There exist various sophisticated data processing approaches for QTOFMS data. Using targeted screening, a reference standard is required to match measured retention time and tandem mass spectrum. However, suspect screening involves relying on information on molecular formula and structure for the tentative identification of compounds present in a sample can be useful when no reference standards are available.
The aim of this work was to demonstrate a workflow for the detection and identification of NPS in serum and urine analysed using data-independent acquisition mode (All-ions MS/MS) on LC-QTOFMS. The data processing workflow combines and structures fundamentals of target and suspect screening data processing techniques in a structured algorithm. This allows the confirmed and tentative identification of compounds. In addition, we applied the workflow to four actual case studies involving poly-drug intoxications.
The LC system consisted of an Agilent 1290 Infinity LC, coupled to an Agilent 6530 Accurate-Mass QTOF MS. The LC gradient separation was performed using a Phenomenex Biphenyl column (100 x 2.1 mm, 2.6 µm). Data-independent acquisition was performed in positive and negative MS mode with 3 scan segments at 0, 15, and 35 eV with fragmentor at 100 V at a rate of 2.5 spectra/s. Acquired data were processed using a workflow algorithm based on post-acquisition data processing strategies linked to an in-house developed database with >1500 entries containing NPS, their metabolites and common product ions. We applied the method to serum and urine samples were prepared using a liquid-liquid extraction at pH 9.5 with hexane: ethyl acetate (7:3) and protein-precipitation with acetonitrile (1:2), respectively.
Using this workflow, we detected and confirmed the parent compounds 4-methoxymethamphetamine (PMMA), ketamine, methiopropamine, flubromazepam, and nitracaine. Additionally, we were also able to tentatively identify pyrovalerone, nor-LSD and in silico predicted phase I hydroxylated and phase II conjugated metabolites for most of the parent compounds detected in the samples whose structures were confirmed by interpretation of accurate MS/MS fragments.
The LC-QTOFMS workflow demonstrates the added value for the detection and identification of NPS and their metabolites in forensic toxicology intoxication cases.

THOP 20 RAPID DETERMINATION OF NEW PSYCHOACTIVE SUBSTANCES IN BIOLOGICAL MATRICES USING AN AUTOMATED IN-LINE ITSP(TM) SPE-LC-MS/MS-SYSTEM

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The number of new psychoactive substances (NPS) on the global market has increased dramatically - in 2014 the European Monitoring Centre for Drugs and Drug Addiction alone registered 101 new substances. The NPS diversity has resulted in a continual analytical challenge.
To develop and validate a high-throughput, simple, robust and fully automated in-line solid-phase extraction-liquid chromatography-tandem mass spectrometry (SPE-LC-MS/MS) method for rapid and simultaneous analysis, in urine and serum, of more than 50 NPS covering the substance classes of synthetic cathinones, phenethylamines, aminoindanes, phencyclidine-type substances, ketamine, tryptamines, piperazines and synthetic cocaines.
50 µL of ammonium formate and a deuterated internal standard mix were added to 150 µL of urine/serum specimens, mixed and then applied to the in-line SPE system Instrument Top Sample Preparation (ITSP Solutions, Inc.). A polymer-based mixed-mode sorbent with strong cation exchange functionalities (Plexa PCX, Agilent) was used for the extraction. The extracts were directly injected onto an LC-MS/MS system, using a biphenyl column and gradient elution with 2 mM ammonium formiate/0.1 % formic acid and acetonitrile/0.1 % formic acid as mobile phases. Data acquisition was performed in multiple reaction-monitoring (MRM) mode with positive electrospray ionization. The method was validated with respect to the guidelines of the Society of Toxicological and Forensic Chemistry (GTFCh), set out according to DIN EN ISO/IEC 17025 regulations. A fully automated SPE method was developed, which delivers the entire process of sample preparation and application. A single extraction step was used for eluting all target compounds from both urine and serum.
The developed method records more than 50 NPS within a total analysis time of 8.3 min per sample, including sample preparation. Limit of quantification (LOQ) is ≤ 8 µg/L in both matrices, with a limit of detection (LOD) ≤ 0.8 µg/L for the majority of analytes (80 %). The method provides good precision and accuracy. Recoveries were in the 50 and 110 % range, and matrix effects ranged between 75 % and 125 %. After validation, the method was successfully applied to routine urine and serum samples.

A highly efficient, fast, selective and sensitive method - capable of detecting a multitude of NPS in biological matrices such as urine and serum - has been developed - with the added advantage of using only 150 µL specimen volume. This technique substantially reduced the total cycle time, by means of interlacing the sample preparation and LC-MS/MS measurement of sequent samples, and significantly improved the forensic-toxicological routine casework process. According to published casework the achieved LODs and LOQs appear to be ideal for routine casework.

THOP 21 QUANTIFICATION OF DESMETHYL METABOLITES BY UPLC WITH CORONA CHARGED AEROSOL DETECTION USING THE PARENT DRUG FOR CALIBRATION

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Many drugs are metabolized through demethylation and some of the desmethyl metabolites are active compounds themselves. The availability of reference standards for many desmethyl metabolites, however, is limited and expensive. Corona Charged Aerosol Detector (CAD) is a universal, mass-dependent detector. The response of CAD depends mainly on the amount, not on the physicochemical properties of the analyte, thus enabling quantification. The aim of this study is to investigate whether the quantification of desmethyl metabolites in blood samples is possible using their parent drugs for calibration when utilizing ultra-high performance liquid chromatograph (UPLC) with the universal CAD detector.
Quantification of metabolites using the parent drugs for calibration was carried out using 24 pairs of parent drugs and their N-desmethyl or O-desmethyl metabolites. Standard solutions (1 mg/mL) were prepared in methanol. Quantification was first performed by analyzing standard solutions. To study the effect of sample preparation, blood samples were spiked with the studied compounds and liquid-liquid extraction was carried out. Internal standard (dibenzepin, 50 µl, 20 µg/mL) and 1M Tris-buffer (300 µl, pH 11) solutions followed by the extraction solvent, ethyl acetate:butyl acetate (1 mL, 25:75, v:v), were added to 1 mL of blood, and the sample was carefully extracted in a multi-tube vortex mixer. After centrifugation (5 min, 4300 g), the organic layer was evaporated to dryness, reconstituted with 100 µl of 0.1% aqueous trifluoroacetic acid:methanol (80:20, v:v). The analysis method was a previously published method utilizing UPLC-diode array detector-CAD (UPLC-DAD-CAD) instrumentation. For quantification with CAD, single point calibration curves were created using the parent drug as calibration standard.
Quantitative results for the desmethyl metabolites were within 20% of the target values for 20 of the 24 metabolites when calibration was performed with the corresponding parent drug. Analysis of spiked blood samples yielded similar results, demonstrating that extraction efficiencies of parent drugs and desmethyl metabolites were comparable. Using a gradient run affected the response on CAD, but since most of the desmethyl metabolites eluted in the close proximity of their parent compound, the change in gradient composition did not have a significant effect on the responses. Even with retention time differences greater than one minute, quantitative results were well within the uncertainty of measurement (30%) of the normally calibrated UPLC-DAD-CAD method.
Quantification of N- and O-desmethyl metabolites in blood by the UPLC-DAD-CAD method, using the parent drug for calibration, is a feasible method when reference standards are not available. The approach provides a cost-effective solution to analyzing parent/metabolite -ratios in real samples, as opposed to mass spectrometric methods that suffer from non-uniform response and matrix effects. As the UV spectra of the N-desmethyl metabolites are very similar to those of their parent drugs, identification of metabolites without reference standards can be based on the UV spectra.

THOP 22 COCAINE AND METABOLITES CONCENTRATIONS IN DRIED BLOOD SPOTS AND VENOUS BLOOD AFTER CONTROLLED INTRAVENOUS COCAINE ADMINISTRATION

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Cocaine is a widely abused central nervous system stimulant worldwide. Dried blood spots (DBS) are an increasingly common clinical sampling alternative to venous blood due to their non-invasive, easy collection, and increased stability; however, there are limited controlled drug administration studies with side-by-side comparison of venous blood and capillary DBS samples.
The aim of the present study was to examine the correlation between DBS and venous blood simultaneously collected from participants following controlled intravenous (IV) cocaine administration. We validated methods for quantifying cocaine and its metabolite, benzoylecgonine (BE), in DBS and venous blood by liquid chromatography-high resolution mass spectrometry (LC-HRMS) and two dimensional-gas chromatography-mass spectrometry (2D-GC-MS), respectively.
In this Institutional Review Board (IRB)-approved study, adult cocaine users were administered single 25 mg IV cocaine doses on Days 1, 5 and 10, after providing written informed consent. DBS and venous blood specimens were collected 30, 60, and 90 min post cocaine administration. DBS were extracted with 1% formic acid in water followed by cation solid-phase-extraction. DBS were analyzed by LC-HRMS with a Thermo Scientific Q-Exactive for cocaine, BE and norcocaine in targeted-MSMS mode to achieve the required lowest limits of quantification. Venous blood specimens were extracted utilizing UCT CleanScreen DAU copolymeric cartridges and derivatized with ethyl

acetate:MTBSTFA+1% t-BDMS (50:50 v/v). Extracts were analyzed by 2D-GC-MS (Agilent Technologies) for cocaine and BE. Norcocaine was not quantified in blood because it chromatographed poorly and did not yield clinically relevant sensitivity. Methods were validated in accordance with the Scientific Working Group for Forensic Toxicology (SWGTOX) Standard Practices for Method Validation in Forensic Toxicology.
Sensitive and specific methods were developed and validated for DBS and venous blood cocaine and metabolite detection with linear ranges from 1-200 µg/L. Acceptable bias and imprecision (±15% target) were achieved. No exogenous interferences were observed at the upper limit of linearity (200 µg/L). DBS and venous blood stability were comparable, with all analytes stable (< -13.2%) under all tested conditions, except cocaine in venous blood at 4°C for 72 h (25% loss). Cocaine and BE were detected in all DBS and matched blood specimens after IV cocaine administration; however, no measureable norcocaine was detected in DBS. Cocaine and BE concentrations in authentic matched specimens' (392 DBS (up to 5 spots per card), 97 venous blood) from 13 participants trended lower in DBS with wider ranges and greater variability (21.4-105.9 %CV) than in blood. DBS %CVs indicated high variability among the 5 spots per DBS card with medians (range) of 22.3% (4.9-122%) for cocaine and 9.8% (0.1-143%) for BE. There was poor agreement between capillary DBS and venous blood cocaine and BE concentrations due to variable DBS collection volume, hematocrit bias and/or lack of sample homogeneity.
DBS offer advantages for monitoring cocaine intake compared to venous blood; however, DBS concentration variability must be addressed to improve the method's clinical usefulness. Supported by the National Institutes of Health, Intramural Research Program, National Institute on Drug Abuse

THOP 23 SUPPORTED LIQUID EXTRACTION (SLE) FOR THE ANALYSIS OF METHYLAMPHETAMINE, METHYLENEDIOXYMETHYLAMPHETAMINE AND DELTA-9-TETRAHYDROCANNABINOL IN ORAL FLUID AND BLOOD

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Since 2006, the South Australian Government has been conducting roadside testing of drivers for the illicit drugs methylamphetamine (MA), methylenedioxymethylamphetamine (MDMA) and delta-9-tetrahydrocannabinol (THC). Forensic Science South Australia carries out the confirmatory analysis by LCMS for the positive test results of oral fluid roadside testing along with the pre-screened ELISA positive road traffic accident blood samples. The number of samples analysed has been steadily increasing (up to 200 samples per week) and so an investigation was launched to streamline the method for the extraction and analysis of the three analytes.
With the possibility of future automation of the extraction procedure, various solid phases available in a 96 well plate format were investigated. The Biotage Isolute® SLE+ 200uL and 400uL plates offered a fast and efficient method of extraction for all three analytes. The aim of this project was to validate a method for the extraction and analysis of MA, MDMA and THC from 100uL buffered oral fluid samples or whole blood using the Isolute® SLE 96 well plates.
Sample preparation included 1:1 dilution with a dilute ammonia solution for buffered oral fluids (1:3 dilution for blood samples), and addition of deuterated internal standards. Samples were loaded onto the phase, left to absorb for 5 minutes then eluted with MTBE. The samples were evaporated and reconstituted in methanol. LCMS analysis was performed on an AB Sciex 5500 Qtrap in positive ion mode, monitoring 3 transitions for each analyte. Separation was achieved on a Restek Ultrabiphenyl 50 x 2.1mm column with a gradient system of acetonitrile / 0.1% formic acid over 5 minutes.
Recoveries above 80% were achieved for MA and MDMA (25ng/mL) whilst recoveries of greater than 65% were achieved for the THC at 4ng/mL.The accuracy and precision were acceptable down to the LLOQ for all three analytes (5/5/1ng/mL for MA/MDMA/THC respectively) with little or no matrix effects for the oral fluid samples. Linearity of greater than R^2=0.999 was achieved for all analytes.
Validation of the SLE extraction and LCMS analysis has allowed rapid sample analysis of oral fluid and blood samples for the drugs in driving program in South Australia. Up to 180 oral fluid samples can be extracted and ready for LCMS analysis within four hours. The method is easily transferable to an automated liquid handling platform.

THOP 24 SIMULTANEOUS DETERMINATION OF 40 NOVEL PSYCHOACTIVE STIMULANTS IN URINE BY LIQUID CHROMATOGRAPHY HIGH-RESOLUTION MASS SPECTROMETRY AND LIBRARY MATCHING

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The emergence of novel psychoactive substances (NPS) is an ongoing challenge for analytical toxicologists. Different analogs are continuously introduced in the market to circumvent legislation and to enhance their pharmacological activity. Although detection of drugs in blood indicates recent exposure and links intoxication to the causative agent, urine is still the most preferred testing matrix in clinical and forensic settings.
We developed a method for the simultaneous quantification of 8 piperazines, 4 designer amphetamines and 28 synthetic cathinones and 4 metabolites in urine by liquid chromatography coupled to hybrid quadrupole high-resolution mass spectrometry (Q-Exactive).
One hundred µL urine was subjected to solid phase cation exchange extraction (SOLA SCX cartridges). The chromatographic reverse-phase separation was achieved with a gradient mobile phase of 0.1% formic acid in water and in acetonitrile in 20 min. The column employed was Accucore C18 100x2.1mm, 2.6µm. Data were acquired in full scan

and data dependent MS2 (ddMS2) mode. Compounds were quantified by precursor ion exact mass, and confirmed by product ion spectra library matching, taking into account product ions' exact mass and intensities (score≥60). Eleven deuterated internal standards were employed.
The assay was linear from 2.5 or 5 to 500µg/L. Imprecision (n=15) was <15.4%, and accuracy (n=15) 84.2-118.5%. Extraction efficiency was 51.2-111.2%, process efficiency 57.7-104.9% and matrix effect ranged from -41.9 to 238.5% (CV<23.3%, except MDBZP CV<34%). Dilution integrity was observed after 1:5 to 1:20 dilutions with blank urine (77.9-104.2% accuracy). Authentic urine specimens (n=62) were analyzed, with 49 positive for piperazines, designer amphetamine and/or synthetic cathinones. Up to 7 different drugs were detected in one specimen, but most were positive for 1 or 2 drugs (n=12 and 11, respectively). Concentrations ranged from 2.7 to >10,000µg/L. Specimens above the upper limit of quantification were reanalyzed diluted with blank urine.
The method provided a comprehensive confirmation for 40 new stimulant drugs with high specificity and sensitivity. The full scan-ddMS2 approach also offers flexibility to include additional NPS with minimal method validation steps saving laboratory time and resources. We showed that many NPS stimulants can be detected as parent compounds in urine. Supported by the Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health.

THOP 25 DEFINITIVE MULTI-ANALYTE DRUG TESTING IN URINE BY UPLC-MS/MS: AN ALTERNATIVE TO PRESUMPTIVE SCREENING BY IMMUNOASSAY

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Drug screening is an essential analytical tool for detection of therapeutic, illicit and emerging drug use. While postmortem drug identification relies heavily on limit of detection screening using definitive methods (GC-MS and LC-MS technology), drug screening in the court, employment and clinical settings is generally performed by immunoassays based on administrative threshold criteria. Replacement of immunoassays with multi-analyte definitive methods of screening has been hampered by complex sample preparation, long chromatography time and ion-source matrix effects.
The aim of this study was to develop and validate a rapid and threshold-accurate urine multi-analyte screen for 31 drugs and/or their metabolites including benzodiazepines, opiates, opioids, cocaine, phencyclidine, amphetamines and cathinones. A novel threshold analyte calibration (TAC) technique was used to achieve threshold-accurate drug detection with UPLC-MS/MS analysis.
A simple preparation of urine (200µL), involving rapid glucuronidase hydrolysis followed by dilution and filtration, was performed in 96-well-plate format followed by direct UPLC-MS/MS analysis of the filtrate. Dual transition-ion monitoring for the detection of drugs and metabolites was optimized in a three minute MS/MS acquisition program following chromatographic separation on an ACQUITY UPLC BEH phenyl column (1.7 um, 2.1 x 50mm). TAC technique involved testing urine with (spiked) and without (neat) reference-analytes spiked at the administrative threshold concentration. The analyte specific thresholds used in the assay ranged from 10 to100 ng/mL. A recovery standard (methapyrilene) was added to neat and spiked samples to verify precision of injection volume. TAC ratio of neat to spiked ion area was determined for each analyzed specimen and was calibrated for each analyte using calibrator urine containing threshold concentration of the analytes.
Validation of accuracy and precision (%of target, %CV) at above and below threshold concentrations was determined by replicate analysis of control urine pools containing 50 (96,8), 75 (99,9), 125 (100,9) and 150 (100,9) percent of threshold concentration. Range of accuracy (91-116% of target) and precision (2-15%) was within 20% for all analytes. Within run coefficient of variation of TAC ratio for calibrators prepared in seven urine pools was within 15% (mean 6.4%; range 3.2-13.6%). Matrix effect studies demonstrated a mean absolute recovery of 83% (range 43-113%) across all analytes, based on transition ion area of analyte-supplemented urine compared to supplemented mobile phase. Within-analyte matrix effect varied significantly between urine specimens and was directly correlated with urine creatinine concentration for earlier eluting analytes. TAC ratio normalized the variable matrix effect on ion recovery and allowed consistent and threshold-accurate detection of analytes. Full concordance with proficiency testing results and with definitive confirmation testing results for de-identified case specimens further demonstrated the accuracy and selectivity of drug and metabolite detection by the definitive method of screening. TAC technique coupled with rapid UPLC-MS/MS analysis offer the potential for definitive drug screening as an alternative to presumptive immunoassay screening for both forensic and clinical applications. The TAC technique of LC-MS/MS screening is also applicable to threshold-accurate detection of rapidly emerging designer drugs where deuterated internal standard are not yet available.

THOP 26 **DETERMINATION OF MEDICAL AND ILLICIT DRUGS IN POST MORTEM DENTAL HARD TISSUES AND COMPARISON WITH ANALYTICAL RESULTS FOR BODY FLUIDS AND HAIR SAMPLES**

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In burnt or skeletonized bodies dental hard tissue is sometimes the only remaining sample material. Therefore, it could be used as an alternative matrix. For forensic interpretation, routes and rates of incorporation as well as differences between tooth root, tooth crown and carious material have to be taken into account.
The aim of this study was to qualitatively and quantitatively analyze medical and illicit drugs in dental hard tissues (root, crown, carious material) and to compare the findings with results in materials routinely analyzed in post mortem toxicology.
In three cases with a history of drug intake one whole tooth was obtained during autopsy. The pulp was removed in two cases; in one case the tooth was root canal treated. The teeth were then separated into carious material, root and crown. The powdered dental materials were extracted with methanol under ultrasonication. The extracts were screened for drugs by LC-MSn (ToxTyperTM) and quantitatively analyzed with LC-MS/MS in MRM mode. The findings were compared to the analytical results for femoral blood, cardiac blood, urine, stomach contents and hair. The substances found in body fluids and hair were mostly detected in dental materials as well. In dental materials, amphetamine, MDMA, opioids (morphine, codeine, norcodeine, methadone, EDDP, fentanyl, tramadol), benzodiazepines (diazepam, nordazepam) and promethazine could be detected. The concentrations declined in the following order (e.g. morphine): carious material (11 pg/mg) > root (2.0 pg/mg) > crown (1.5 pg/mg). In contrast, the root canal treated tooth showed higher concentrations in the crown than in the root. Cocaine, benzoylecgonine and cocaethylene were only detected in hair and could not be detected in dental material. On the other hand, codeine was detected in femoral blood and tooth material, but not in hair.
The analysis of dental hard tissue may represent a useful alternative matrix for post mortem toxicology, especially if there is no other material available. Based on our study, the incorporation of medical and illicit drugs into dental hard tissue depends on physico-chemical properties of the substance and seems to take place mainly via blood. The enamel can be regarded as a protecting barrier preventing a significant incorporation of drugs into the crown dentin via oral fluid. The preliminary results of this study suggest a window of detection for tooth material located between the detection windows known for blood samples (hours to days) and hair samples (weeks to months).

4.30-6.00 pm SESSION 4 – New Psychoactive Substances II (abuse patterns and identification)

THOP 27 DESIGNER STIMULANTS - EVOLVING ABUSE PATTERNS, 2011-2015

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An avalanche of designer substances has been coming to the recreational drug market in the recent years. Their availability and abuse patterns have been constantly changing following regulatory actions, effective control by drug testing laboratories and respective response by designer drug industry. However, a detailed picture of timing, scale of abuse and succession of individual drugs has not been readily available.
The aim of this study was to present retrospective laboratory data on the detection and prevalence of individual designer stimulants (35 drugs, mostly synthetic cathinones) in routine testing of about 100000 human urine samples over a period of the last 4-5 years. We also provide recommendations on the analysis and on the choice of appropriate analytes (parent or metabolite).
Un-conjugated stimulants were isolated from urine by liquid/liquid extraction in alkaline conditions and then derivatized with trifluoroacetic anhydride. Analysis was performed by GC/MS.
Substituted cathinones were the most frequently found designer stimulants. Over time they succeeded each other in the following order: methylenedioxypyrrovalerone (MDPV), methylone, pentedrone and α-pyrrolidonopentiophenone (PVP). A very large number of less frequently detected cathinones (and their metabolites) was observed in 2011 and early 2012. At the same period, positivity rate for synthetic cathinones reached 8-12% of the total samples tested. This was roughly equal to methamphetamine/ amphetamine positivity in the same population. After that both positivity (2%) and variety of cathinones declined sharply with PVP remaining the most prevalent. Generally, the decline of the drug detection followed government scheduling. Various exotic cathinones are still detected on a low level, including the ones, which have not been previously seen. Positivity rate for traditional designer stimulants, like MDMA and BZP, was very low 0.025% and it did not change over time. Various fluoromethamphetamines and fluoroamphetamines were relatively abundant (up to 0.5% positivity) in 2012, then declined sharply and have not been seen lately. The data will also be presented for the thiophenyl substituted methamphetamine (MPA), PMMA, 5- and 6-APB, 2CE, 2CD, ethylphenidate, "Craze" (2-ethylamino-1-phenylbutane) and methoxethamine.
Timing, extent of abuse and succession of individual designer stimulants in the US are presented. Initially high (10%) positivity have dropped to 2% in 2013-2015. 20 different synthetic cathinones were detected in routine testing of about 100,000 urine samples. Rise and decline trends in the use of individual drugs have been observed. Positivity rate for the classic designer stimulants (MDMA, BZP, PMMA, 2C- drugs) was negligible.

THOP 28 TRENDS AND CHANGES IN THE SYNTHETIC CANNABINOID DRUGS MARKET IN ISRAEL BETWEEN 2010-2014

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Around 2010, synthetic cannabinoid products have become a critical phenomenon in the drug realm worldwide. In Israel, as in many other countries, the rapid widespread and easy availability of these drugs, along with their health hazards and addiction potential, caused alarm among state authorities and the general public. In Israel, these concerns lead to the rapid introduction of synthetic cannabinoids into the Controlled Substances Act, and, in 2014, to an extensive campaign against their use.
The aim of the present study was to analyze the influence of scheduling of synthetic cannabinoids on their abuse pattern among the Israeli young population (18 to 22 years old). The impact of the public campaign on the trends of drugs of abuse consumption among youngsters was also examined
The results of analysis of 14,000 forensic exhibits seized from young people suspected of drugs abuse, and of 11,000 forensic urine samples tested in the years 2010-2014 were collected. The relationship between the types of drugs found in the samples and the scheduling of the drugs was analyzed retrospectively. Exhibits were tested by gas chromatography-mass spectrometry in a general unknown screening mode. Urine samples were tested by immunochemistry followed by gas chromatography-mass spectrometry confirmatory tests for: cannabis, cocaine, opiates and amphetamines. Because of technical limitations, urine samples were not tested for synthetic cannabinoids.
The laboratory tests approximately 3,000 exhibits per year, seized from young people. Percentage of exhibits found to contain cannabis dropped from 50% in 2010 to 38%, 33% and 20% in 2011, 2012 and 2013, respectively. Interestingly, an increase of cannabis-containing exhibits (29%) was observed in 2014. The first synthetic cannabinoids found in exhibits (by mid-2010) were JWH-073 and JWH-018. In 2011, along with scheduling of these substances, their incidence in exhibits dropped and AM-2201 appeared. Similarly, in May 2012 AM-2201 was scheduled and almost disappeared from exhibits, while UR-144, XLR-11 and MAM-2201 were found. In 2013 we detected PB-22, AB-PINACA and AKB-48. Synthetic cannabinoids typical of 2014 were AB-CHMINACA and AB-FUBINACA (scheduled further on in 2014). The increase in the number of cannabis-positive exhibits in 2014 coincides with an increase in percentage of cannabis-positive urine samples (13.6% in 2014 compared with 7.7% in 2010, 9.8% in 2011, 9.3% in 2012, and 6.2% in 2013). Results showed that the progressive introduction of synthetic cannabinoids into the Controlled Substances Act caused concomitant shifts in the abuse pattern of the young population. Abuse of synthetic cannabinoids also affected the abuse of cannabis. In 2014, there seems to be a return to cannabis, probably due to extensive scheduling of the synthetic substances, and possibly to the impact of the public campaign stressing their hazard to health. Following trends in 2015 will enable us to assess whether we are observing a new trend, or a casual fluctuation.

THURSDAY, September 3rd

THOP 29 NEW PSYCHOACTIVE SUBSTANCES: THE PERKS AND PITFALLS OF ON-LINE PURCHASING

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New psychoactive substances (NPS) (designer drugs, research chemicals, "legal highs") have been emerging onto the recreational drug scene at an unprecedented rate. In parallel the number of substances notified to the EU Early Warning System (EWS) for the first time is increasing year on year, and shows no signs of abating. The correct identification and quantification of these compounds in case work is dependent on the availability of appropriately certified reference materials, but toxicologists and reference material producers alike are floundering under the ingenuity of the market, and the speed at which it reacts. Whilst synthesis is considered the 'gold standard' for reference material production the time frame and cost associated with this fails to meet the current demand, and new strategies are needed to keep pace with this rapidly evolving market. On-line purchasing from research chemical vendors, known as 'grey market sourcing', may not seem the most ethical of routes, but enables delivery of affordable reference materials in a timely fashion. However, such an approach is not without its pitfalls and warrants extreme caution in both product sourcing and material verification. Here we share our experience of some of the 'perks and pitfalls' of on-line purchasing.
To investigate the effectiveness and reliability of on-line purchasing as a complementary, fast and cost reducing approach to in-house synthesis of certified reference materials for NPS.
The Internet acts as a vast shop window for NPS. In 2013 the EMCDDA identified 651 websites marketing legal highs to Europeans. Chiron regularly monitor a range of on-line retailers as well as drug discussion forums for new product inspiration. On-line availability correlates well with requests made to reference material producers. To meet current demand for quicker and cheaper reference material production Chiron embarked on a programme of on-line purchasing. The products were analysed, purified where required, and certified to the same standard as material synthesised in-house.
In our experience on-line sourced materials are more often than not what they claim to be. Products are almost always marketed as free base, but may salt form (i.e. 2-AI, 6-EAPB, Nitracaine etc). Purity is typically >90% and often >98%, however traditional methods for determining purity and identity (HPLC, GC, NMR, IR, Karl Fischer) could overlook inorganic content. Ash content should be investigated as a matter of routine.
There are undoubtedly advantages in grey-market sourcing, however, it should not be attempted without close cooperation with customs authorities, in particular if sourcing oversees. Irrespective of intended use, products can only be sourced within the confines of the law. On-line sourced products are not fit for use as reference materials 'as is'. They require analysis, purification and certification using a wide spectrum of specialist analytical techniques. Lack of confidence in deliverability from supplier is a significant challenge.

THOP 30 FAST IDENTIFICATION OF NEW PSYCHOACTIVE SUBSTANCES IN COLLABORATION WITH EUROPEAN CUSTOMS

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New Psychoactive Substances (NPS) have seen a dramatic increase over the past years as abused substances. Customs and forensic laboratories are faced with the challenge of quickly identifying these compounds. As the first rampart of the European Union, customs laboratories are responsible for the control of products entering this market.
The European Commission, Joint Research Centre (JRC) and Directorate General Taxation and Customs Union (DG TAXUD), are providing support for the control of new NPS seized by European Customs. Efficient tools for rapid and unambiguous identification of new designer drugs are needed to enable fast decisions regarding imported substances. Full chemical identification can be a lengthy procedure and requires highly sophisticated analytical instrumentation. The JRC High Resolution Nuclear Magnetic Resonance (NMR) and Mass Spectroscopy (MS) laboratory supports the customs laboratories for the chemical identification of unknown substances.
Multinuclear (1H, 13C, 15N, 19F) mono and bi-dimensional NMR (600 MHz) experiments are performed on samples diluted in d6-DMSO (10 mg/600 μL). The hypothetical structures are then submitted to fragmentation prediction using Mass Frontier® v 7.0 software and then compared with experimental HR-MS(/MS) data. The determination of the exact mass and fragmentation experiments are done using a Q-TOF and/or LTQ-Orbitrap hybrid mass spectrometers. The HR-MS(/MS) experiments are performed on 2 μg/mL solutions in 50/50 MeOH:H_2O + 0.1 % FA. Analysis using FT-IR is done in the powder sample. This analytical strategy allows the establishment of the chemical structure with a very high level of confidence.
Samples were characterised by NMR, HR-MS and FT-IR at the JRC within 48 h and the final structure confirmed.
The JRC, as the European Commission scientific support service, can play a key role in the fast and reliable characterisation of non-classified and unknown substances seized by European Customs. Its contribution could assist the European Commission in evidence based policy making in this field.

THOP 31 IN SILICO PREDICTION AND IN VITRO METABOLISM STUDIES OF NEW DESIGNER DRUGS 25I-NBOME AND 25I-NBOH

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During the last decade the illicit drug market of novel psychoactive substances (NPS) has rapidly grown, and one important group has recently emerged, the N-methoxybenzyl (NBOMe) derivatives of substituted phenethylamines. NBOMes have originally been developed as positron emission tomography (PET) radiotracers, but as the compounds are potent serotonin 5-HT2A receptor agonists, they mediate hallucinations and have been subject to abuse. Abuse of these drugs has been known since 2011, and several non-fatal and fatal intoxication cases have been reported. Especially the abuse of 25I-NBOMe has been linked to deaths. Due at least in part to the latter and also legislative pressure, a new variant group of N-hydroxybenzyl derivatives (NBOH) of substituted phenethylamines have emerged in internet fora, as alternatives to the NBOMe group of compounds. Little is known about the pharmacokinetics of the NBOMes and NBOHs and their metabolic fate. When developing new analytical methods for detection of these NPS in blood or urine, it is of relevance to know the metabolism, since metabolites may be more readily detected than the drug itself.
The aim of the present study was to determine the metabolic fate of 25I-NBOMe and 25I-NBOH in vitro and compare the results with in silico predicted sites of metabolism. Furthermore, to evaluate the risk of toxicity in populations with genetic variations of metabolic enzymes, the aim was also to identify the individual enzymes involved.
25I-NBOMe and 25I-NBOH were incubated with pooled human liver microsomes (HLM), pooled cytosol, pooled S9 fraction, and recombinant cytochrome P450 (CYP) enzymes and monoamine oxidases (A and B). The incubations with HLM and S9 fraction contained co-factors for CYP and also glucuronosyltransferase (UGT) in some experiments. The incubation with cytosol contained co-factors for alcohol dehydrogenase and aldehyde dehydrogenase. Negative controls (without co-factor) where included for all incubations. All samples were analyzed by HPLC-MS/MS.
Initial results from incubation with HLM including the co-factor for CYP (NADPH), showed that 25I-NBOMe and 25I-NBOH shared the same biotransformation pathways. The reactions included hydroxylation, demethylation, N-dealkylation, and dehydrogenation. The compounds were stable in the negative control experiments (no co-factor added). The most abundant biotransformation for 25I-NBOMe and 25I-NBOH were monohydroxylation at the N-methoxybenzyl and N-hydroxybenzyl part of the compounds, respectively, followed by demethylation. Low activity was observed in the incubations with cytosol and MAO. A glucuronidated metabolite of 25I-NBOH was observed in the incubation with HLM and UDPGA (co-factor for UGT). No activity was observed for 25I-NBOMe under the same experimental conditions.
The investigated compounds, 25I-NBOMe and 25I-NBOH, were primarily metabolized by CYP enzymes, resulting in hydroxylated and demethylated metabolites. 25I-NBOH was also subject to direct glucuronidation. Further studies are planned to identify the individual CYP enzymes being involved.

THOP 32 25I-NBOME - CASE REPORT AND METABOLISM

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In recent years a number of so called 'NBOMes' emerged on the drug market. This drug class consists of a phenethylamine backbone (e.g. a representative of A. Shulgin's '2C' series) carrying a 2-methoxybenzyl substituent at the amino nitrogen. This modification is known to enhance the psychotropic effects of the phenethylamine. We report on a 42 year-old man who took a sip of a paediatric analgesic syrup containing an ethanolic solution of 25I-NBOMe deposited in the fridge by his son (supposed 25I-NBOMe concentration: 320 µg/ml). Thirty minutes later he was presented to the emergency department and shortly after transferred to the intensive care unit with severe agitation and auditory as well as visual hallucinations. After six hours the symptoms resolved and the patient was discharged without further complications on the next day. An urine and a serum sample as well as the ethanolic solution were collected and analyzed with LC-ESI-MS/MS.
The aim of this work was to determine the drug concentration in the samples and to elucidate the human metabolism using an in vitro assay based on pooled human liver microsomes (pHLM). The analytical results will improve the data basis for evaluating drug concentrations in clinical and forensic cases.
Sample preparation for the urine and serum sample consisted of an automated solid phase extraction. The ethanolic solution was diluted with ethanol prior to analysis. For the metabolic studies a 25I-NBOMe solution was incubated with pHLM. Analysis of all samples was performed on a Shimadzu HPLC system coupled to an AB-Sciex QTrap 4000 mass spectrometer.
In the serum sample taken one hour post ingestion, 2C-I and 25I-NBOMe were detected at concentrations of 290

ng/ml and 2.6 ng/ml, respectively. In the urine sample 490 ng/ml 2C-I were detected. Additionally, quantitation of the ethanolic solution revealed a concentration of 2,800 µg/ml 25I-NBOMe. This unexpectedly high amount of 25I-NBOMe probably results from a dosing error of the patient's son. The pHLM approach showed O-demethylation and hydroxylation to be the major metabolic pathways, while a metabolic breakdown to 2C-I was observed, however, to a lesser extent.

The results of the in vitro study indicate that 25I-NBOMe is not primarily metabolized to 2C-I by CYP450 enzymes. Instead, the high 2C-I concentrations could be explained by a degradation catalyzed by other enzymes. Therefore, the clinical effects of an oral uptake of NBOMe compounds could be caused in part by the phenethylamines produced by N-dealkylation. In the literature further cases of 25I-NBOMe intoxications are described, but only few report 2C-I concentrations. The 25I-NBOMe serum level in our case was in the same range as in these reports, thus confirming that even in cases of severe intoxication concentrations in the low ng/ml range can be expected. Compared to the literature, the relatively high urine 2C-I concentration in our case could be explained by the time span between intake and acquisition of biological samples and different routes of administration (oral ingestion vs. sublingual application).

8.30-10.30 am SESSION 1 – New Technologies II

FOP 1 BRAIN DISTRIBUTION STUDIES OF ANTIPSYCHOTIC DRUG IN MOUSE WITH MALDI-TOF MS IMAGING: HALOPERIDOL

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Matrix-assisted laser desorption-ionization-time-of-flight (MALDI-TOF) mass spectrometry imaging is used recently to confirm precise tissue distribution of drugs. To study the brain changes caused by antipsychotic drugs, it is important to investigate the brain precise distribution of antipsychotic drugs. Then, MALDI-TOF MS imaging is very useful for the brain distribution studies
Haloperidol (HP) is a prototypical first-generation antipsychotic drug and is still widely used, but its use causes a number of motor side effects, collectively termed extrapyramidal syndromes. This relates to its structural similarity with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) which is a neurotoxic compound via its pyridinium metabolite MPP^+. Haloperidol is also oxidized to its pyridinium metabolite HPP^+ which is similar in structure and toxicity to MPP^+. In the present study, we treated with haloperidol to consider the neurodegenerative side-effects, and examined the precise distribution of HP and HPP^+ in the mouse brain with MALDI-TOF MS imaging.
HP and HPP^+ are intraperitoneally injected to male ICR mice at 20 mg/kg and the animals were sacrificed 60 min after the drug administration. Then, brains were quickly removed and snap-frozen in dry-ice-cooled hexane at -60°C for 1 min. Each frozen brain was cut with a cryostat (CM3050S, Leica) at -20°C. Tissue sections of 16μm thickness were deposited onto stainless steel plates. After the matrix solution (α-cyano-4-hydroxy cynamic acid,CHCA) was homogenously sprayed onto the brain section with a TM-Sprayer, MALDI-TOF MS imaging was performed. The used MALDI-TOF MS imaging instrument system was an Autoflex II equipped with a Smartbeam-1 and ultrafeXtreme with a Smartbeam-II/Flash-Detector (Bruker Daltonics).MS images were recorded using m/z 376/165 (HP) and m/z 354/165.5 (HPP^+), respectively.
Mouse brain distributions of Haloperidol (HP) and its neurotoxic pyridinium metabolite HPP^+ were examined with MALDI-TOF MS imaging. Results showed that 60 min following i.p. HP, its neurotoxic metabolite HPP^+ was concentrated specifically in the striatum and hippocampus area.
In this study, we examined the quantitative distribution of the neurotoxic pyridinium metabolite HPP^+ obtained from HP in a mouse model. MALDI-TOF MS imaging showed the interesting results.

FOP 2 A MULTICOMPONENT UPLC-Q-TOF METHOD FOR SCREENING OF PHARMACEUTICAL COMPOUNDS IN POST MORTEM BLOOD- VALIDATION, DATA MINING STRATEGIES AND RESULTS FROM ROUTINE ANALYSIS.

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The Norwegian Institute of Public Health annually analyses approximately 1600 post mortem blood samples from legal autopsy cases. From the forensic toxicological analysis of these cases important information regarding the cause of death can be found, whether it is suspicion of intoxication, presence of illegal drugs or lack of compliance with prescribed drugs.
The aim of this study was to develop and validate a multicomponent UPLC-Q-ToF method for screening of 62 pharmaceutical compounds and 4 internal standards in post mortem blood, with the intention to perform a wider non-validated screening using qualitative data analysis in addition.
The sample preparation was performed using protein precipitation (acetonitrile/methanol, 85/15, v/v) followed by clean-up on Supelco SPE-PL columns for removal of phospholipids. Chromatographic separation was achieved on a Waters HSS T3 1.8 µm 2.1x100 mm column in 17 minutes using an Agilent Infinity 1290 series LC-system. Detection was performed in positive mode using an Agilent 6550 quadrupole time of flight mass spectrometer. Validation was performed on both spiked and real samples, using the Agilent MassHunter quantitative and qualitative software (MH Qual and MH Quant). The Broecker, Herre & Pragst's database (PCDL) was applied using Find by Formula (FbF) and Find by AutoMSMS (FbAMSMS) data mining algorithms.
The average peak height repeatability of the 62 targeted compounds was found to be in the range of 0.7-49% relative standard deviation (RSD) with an average of 5.8% in the lower range of the calibration curve and 0.5-6.7% with an average of 1.9% in the upper range. Good maintenance control was found to be critical to achieve reproducible results. The method validation showed that MH Quant calculations were preferred for low abundant compounds. Reproducible results using FbF or FbAMSMS algorithms were found to require higher abundant compounds, depending on use of MS or MS/MS-data. The MH Quant calculations extracts predefined masses, while MH Qual uses data mining algorithms more depending on thresholds, and thus different cut-offs were established. Problems with saturated peaks resulting in large mass error were also observed during the method validation. In MH Quant saturation caused split peaks, resulting in poor concentration estimates. In MH Qual saturated peaks were observed to result in a missed-match against the PCDL. Different criteria for the accepted mass error were applied in order to overcome this problem. However, the mass error limits should be restricted to avoid too many false positives.
A Multicomponent UPLC-Q-ToF method for screening of 62 pharmaceutical compounds in post mortem blood has been validated and successfully applied to real samples. The 62 compounds were quantified and confirmed using other analytical methods. Compounds identified in the qualitative search, and found relevant to the specific case, were also confirmed and quantified.

FRIDAY, September 4th

FOP 3 HIGH SENSITIVITY/SPECIFICITY DETERMINATION OF CARBOHYDRATE-DEFICIENT TRANSFERRIN (CDT) BY A NEW METHOD BASED ON HPLC SEPARATION WITH FLUORESCENCE DETECTION

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Although CDT has since long been established as one of the most important biomarkers of chronic alcohol abuse, on the analytical side little innovation has appeared in the latest decade. In particular the current instrumental technologies are still limited to HPLC and capillary electrophoresis, both using light absorbance detection at 460 and 200 nm respectively. These two detection wavelengths show distinct advantages and disadvantages, being the molar absorptivity of transferrin at 460 nm extremely low, and the absorbance at 200 nm common to all proteins, peptides and large number of organic compounds. On the other hand, fluorescence detection might offer higher sensitivity and selectivity, but its direct application to transferrin analysis is hampered by a lack of specificity, since all the other serum proteins share the same fluorophores (mainly tryptophan). On these grounds, it is clear that the important advantages of fluorescence detection of CDT in terms of sensitivity and specificity can be exploited only after a specific fluorescent labelling of transferrin.
Aim of the present work, carried out in the frame of the SafeDrive Project (funded by the Department of Antidrug Policies, Rome), was to develop a method for the fluorescent labelling of transferrin and an efficient HPLC_FL method for the separation and detection of the CDT related glycoforms.
Human serum specimens were diluted (1:5) with aqueous Tb^{3+} salt solution and directly injected in HPLC (5 microL). The fluorescence detection (λex 280nm – λem 550nm) was carried out by HPLC with a double monochromator fluorimeter. On the separation side, HPLC used an anion exchange column (4.6 x 100 mm, particle size 5 micrometers, Waters Protein Pack) eluted with a linear NaCl (0.5 M) gradient in Tris buffer (20 mM pH 8.1). The fluorescence signal was analysed with a standard chromatographic acquisition software.
Because of Tb^{3+} affinity for the Fe^{3+} binding sites on the transferrin molecule, and the peculiar fluorescence of the resulting complex, excellent selectivity was obtained for all the transferrin glycoforms, which could be easily separated by HPLC. The high fluorescence of Tb-transferrin and the lack of any spurious signal from the biological samples offers excellent sensitivity (at least 10 folds better than in HPLC with absorbance detection). Another advantage of the Tb derivatization of transferrin is that no interference from hemoglobin on the CDT signal was observed. A complete validation of the new method according the requirements of Analytical Chemistry is in progress.
The new method* based onto a specific derivatization of transferrin with Tb^{3+} and its specific fluorescence detection offers new perspectives of sensitivity and specificity to the analysis of CDT. A neat advantage of the described new technique is the possibility of analysing cadaveric blood in which the post mortem haemolysis hampers the application of the current HPLC and electrophoretic methods. *Patent MI2014A000395 and PCT/EP2015/054896

FOP 4 A PROPOSAL FOR THE ORBITRAP-BASED LC-HR-MS/MS STANDARD URINE SCREENING APPROACH: TRANSFER TO BLOOD PLASMA EXEMPLIFIED FOR CARDIOVASCULAR DRUGS

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Urine and plasma are often used for systematic toxicological analysis. In contrast to urine with longer detection windows, plasma shows the current drug intake of a patient.
The aim of the study was to transfer the LC-HR-MS/MS standard urine screening approach to blood plasma.
Human plasma samples (250 µL each) spiked with the drugs for validation and authentic samples submitted for toxicological screening were prepared by precipitation with 750 µL $ZnSO_4$ solution (35 mg/mL water:methanol, 70:30)., or by on-line extraction using turbulent flow chromatography (TFC) according to Helfer et al. (JOC-A, 2014). After separation using a TF Accucore PhenylHexyl (100 x 2.1 mm, 2.6 µm) analytical column and gradient elution, analyte detection was performed using a TF Q-Exactive HR mass spectrometer with a HESI-II source with pos/neg switching. Identification using TF Trace Finder software 3.2 was based on precursor accurate mass, isotopic patterns, 5 most intense fragment ions, and the new reference library (Maurer/Helfer/Meyer/Weber, 2015).
Sufficient separation was achieved in 10 min. The approach was validated as qualitative assays according to Peters et al. FSI, 2007. The results for 20 selected cardiovascular drugs were as follows: Recovery (%): 88-123 for precipitation, 30-132 for TFC Matrix effect (%): 19-125 for precipitation, 22-118 for TFC Process efficiency (%) 19-110 for precipitation, 21-142 for TFC Limit of detection (ng/mL): 0.1-100 for precipitation and for TFC Mass spectral data of 72 cardiovascular drugs (ACE inhibitors, antiarrhythmics, anticoagulants, beta-blockers, diuretics, sartans, fibrates, statins, etc.) and over 200 their metabolites were recorded and added to the library containing currently data of 1,400 drugs and 1,100 metabolites. So far, the method was applied successfully for toxicological screening of more than 500 plasma samples. Among the tested 72 drugs, only cardiac glycosides, NO releasing drugs, dihydralazine, fibrates, and statins could not be monitored after therapeutic intake.
The LC-HR-MS/MS standard urine screening approach was successfully transferred to blood plasma. TFC provided slightly higher sensitivity than simple precipitation. Both extraction approaches allowed fast, robust, reliable, and highly specific detection of all studied cardiovascular drugs and/or their metabolites after regular therapeutic doses. This approach will be transferred to other drug classes.

FOP 5 **UPLC-MS/MS METHOD FOR THE SIMULTANEOUS DETERMINATION OF 35 SUBSTANCES INCLUDING MEDICINES AND DRUGS OF ABUSE ON DRIED BLOOD SPOTS APPLIED IN FORENSIC TOXICOLOGY**

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Dried blood spot (DBS) sampling is a well established small blood volume collection technique used mainly for newborn screening purposes. More recently, this sampling technique has increasingly gained interest for its use in therapeutic drug monitoring, toxicokinetic and clinical studies, as well as in toxicology. Advantages associated with the DBS sampling technique of particular importance for its application in toxicology include its ease of use and minimal invasiveness, the low biohazard risk, the simple transport and sample storage and the stabilizing effect of DBS.
The purpose of the present study was to develop and validate an ultra performance liquid chromatography - tandem mass spectrometry method that allows the simultaneous identification and quantification of 35 substances, including medicines such as benzodiazepines, antipsychotics and antidepressants, and drugs of abuse, namely amphetamines, opiates, cocaine, methadone and metabolites in DBS. After validation, the procedure should be applied to real samples collected in routine forensic analysis and compared to the results obtained with existing validated whole blood methods.
Whole blood samples were spotted onto Whatman BFC180 cards and dried. The whole blood spot was punched from the card and extracted with methanol/acetonitrile (3:1, v/v). The chromatographic separation was performed with an Acquity UPLC® HSS T3 (100 x 2.1 mm i.d., 1.8 µm) reversed-phase column using an acetonitrile/2 mM ammonium formate buffer pH 3.4 gradient and the MS/MS detection was achieved with two precursor-product ion transitions per substance. The method was validated according to international guidelines. Additionally, a storage stability study was performed at two concentration values and using three different temperatures: room temperature, 2-8ºC and -10ºC for a period of time of 5 months.
Specificity was evaluated by analysis of 40 blank blood samples. No interfering peaks were observed in the retention time of the analytes. The extraction efficiency ranged from 42 to 99% and no carryover was observed. No significant matrix effect was observed for all the substances, except for chlorpromazine, sertraline, nortriptyline and morphine with moderate ion suppression and bromazepam and alprazolam with considerable ion enhancement. The limits of detection and quantification ranged from 0.2 to 5 ng/mL and 1 to 5 ng/mL, respectively. The method was linear in the range 1-500 ng/mL. The intra-assay precision was always ≤15%. The inter-assay accuracy resulted in mean relative errors ranged from -11% to 9.8% and inter-assay precision ranged from 4.7% to 14%, respectively. For the stability study, the samples were considered stable with results within ± 15% of the baseline values for the three temperatures. Until now, the method was applied to 25 real samples, with positive results for amphetamines, opiates, cocaine, benzodiazepines and antidepressants showing promising correlation with the whole blood methods.
An UPLC-MS/MS method for the simultaneous determination of 35 substances (including medicines and drugs of abuse) on DBS was developed and validated. The method has demonstrated that the DBS sampling could represent a very useful technique for the collection, assaying and storage of samples in the context of forensic toxicology.

FOP 6 **MICROWAVE-ASSISTED ON-SPOT DERIVATIZATION FOR THE GC-MS BASED DETERMINATION OF POLAR LOW MOLECULAR WEIGHT MOLECULES -AMONGST WHICH GHB AND BHB- IN DRIED BLOOD SPOTS**

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Gamma-hydroxybutyric acid (GHB) is a well-known illicit club and date-rape drug. Dried blood spot (DBS) sampling is a promising alternative for classic venous sampling in cases of (suspected) GHB intoxication since it allows rapid sampling and convenient storage and transport. DBS can also be used as a convenient sample preparation strategy. Determination of beta-hydroxybutyric acid (BHB) is relevant in forensic toxicology since increased levels of this ketone body -typically owing to uncontrolled diabetes or after a history of excessive alcohol abuse- are found in cases with fatal ketoacidosis.
The aim of this study was to evaluate the applicability of a GC-MS method, developed for the simultaneous determination of polar low molecular weight molecules -amongst which GHB and BHB- in DBS. To this end, we applied our method on spots obtained from postmortem samples for the determination of BHB. In addition, the method was also applied on a series of GHB positive cases.
After punching a 6-mm disc from a DBS and adding the internal standard GHB-d6, the punches were subjected to microwave-assisted on-spot derivatization by direct application of 25µL acetic anhydride and 25µL pyridine and heating (90s, 800W), followed by a second derivatization with 25µL heptafluorobutanol (90s, 800W). Following evaporation, the extract was reconstituted in 100µL ethylacetate. One µL of the derivatized extract was injected into an Agilent 6890-5973 GC-MS system. Chromatographic separation was achieved on a 30m x 0.25mm i.d. x 0.25µm Agilent HP-5MS column. Quantification of BHB, GHB and GHB-d6 was performed in SIM mode using m/z 227, 268 and 285 for BHB, 227, 240, 268 and 285 for GHB and 231, 245, 273 and 291 for GHB-d6. Given the different spreading of postmortem (lysed) blood on filter paper, we slightly modified the above-mentioned protocol for the analysis of postmortem samples: a fixed volume (15µL) was spotted on a 7-mm pre-punched disk instead of using a fixed-diameter partial punch.
Calibration lines were linear over the 10-100µg/mL and 5-300µg/ml concentration range for GHB and BHB, respectively, applying 1/x weighted linear regression for both compounds. Precision and accuracy met acceptance criteria

(<20% at LLOQ, <15% at 3 other QC levels), except for BHB, where the bias lay below 19% for 2 QC's. Stability studies revealed no significant decrease of BHB and GHB in DBS. Retrospective analyses on a set of postmortem samples revealed pathologically significant BHB concentrations (>250mg/L) in several cases. The results of the correlation of BHB with acetone, isopropanol and known history of alcohol abuse and diabetes will be presented, in addition to the results of a series of GHB positive cases.
We successfully validated microwave-assisted on-spot derivatization for the GC-MS-based determination of BHB and GHB in DBS. The determination of BHB and GHB proved to be reliable, fast and applicable in routine toxicology, as exemplified by analysis of routine samples .

FOP 7 **DETERMINATION OF SYNACTHEN® IN DRIED BLOOD SPOTS FOR DOPING CONTROL ANALYSIS USING LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY**

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Dried Blood Spot (DBS) sampling, a technique for taking whole blood samples dried on filter paper, was initially reported in 1963 by Robert Guthrie. While the diagnostic analysis of metabolic disorders in newborns was the focus of investigations at that time, the number of established applications for preclinical drug development, toxicological studies and therapeutic drug monitoring increased enormously in the last decades. As a consequence of speed, simplicity and minimal-invasiveness DBS may be a preferential technique in sports drug testing.
The aim of this study was to develop a screening assay for the analysis of the synthetic human adrenocorticotropic hormone, tetracosactide hexaacetate (Synacthen®), in DBS using liquid chromatography tandem mass spectrometry.
A quantitative method was developed using nano-ultra high performance liquid chromatography (nano-UPLC) coupled to high resolution, high accuracy tandem mass spectrometry (HR-MS/MS). In contrast to previously published methods applying immunological assays, the method outlined here permits high specificity without losing sensitivity. Immunoaffinity purification within the sample preparation procedure provides an additional criterion for assay specificity. The identification and determination of the analyte was conducted in targeted selected ion monitoring mode (tSIM) and additionally data dependent MS/MS (ddMS/MS).
Highly purified sample extracts were obtained by an advanced sample preparation procedure including the addition of an internal standard (d8-tetracosactide) and immunoaffinity purification. The method's overall recovery was 27.6% and the assay's imprecision was calculated between 8.1% and 17.9% for intraday and 12.9% to 20.5% for interday measurements. Stability of the synthetic peptide in DBS was shown for at least 10 days at room temperature and presents a major benefit, since a rapid degradation in conventionally applied matrices such as urine or plasma is well known. With a limit of detection of 50 pg/mL, a detection window of several hours is expected considering reported steady-state plasma levels of 300 pg/mL after intramuscular application of Synacthen® Depot (1 mg). The analysis of authentic DBS samples within the scope of an administration study with Synacthen® 250 µg (short stimulation test) demonstrated the great potential of the developed assay to simplify the analysis of Synacthen® for doping control purposes.
The present approach suggests the first application of DBS as sample matrix for the analysis of Synacthen® in doping control analysis. A fast and robust assay providing reliable results was developed and validated. Further, due to its high sensitivity, it is considered to be appropriate for the simultaneous qualitative and quantitative determination of Synacthen® in sports drug testing.

FOP 8

DEVELOPMENT OF A WORKFLOW FOR NON-TARGETED HIGH RESOLUTION ACCURATE MASS SPECTROMETRY ANALYSIS IN FORENSIC TOXICOLOGY: A CASE STUDY IN NBOME DETECTION

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The potential for Liquid Chromatography-High Resolution Accurate Mass (LC-HRAM) technology to identify 'unknown' compounds using non-targeted screening methods provides a tactical advantage in the fight against doping in sport. This innovation comes with the requirement for assessment to support its use in the medico-legal context. Using the example of N-methoxybenzyl (NBOMe) compound detection in equine urine, the aim of this study was to develop a workflow for LC-HRAM hardware combined with differential analysis and in silico modelling software platforms to identify 'unknown' responses and perform structural elucidation.

Six NBOMe compounds (25-B, 25-C, 25-D, 25-E, 25-H and 25-I) were studied to develop and optimize the proposed non-targeted screening workflow before a seventh NBOMe (25-N) was used as a blind control for verification. Blank and adulterated equine urine samples (1.5 mL) were subjected to enzyme hydrolysis prior to solid phase extraction (SPE) using high-flow mixed-mode C8/benzyl sulfonic acid XTRACKT® (200 mg / 3 mL) columns (UCT, Bristol, PA, USA). Basic eluents were submitted to LC-HRAM analysis using an Ultimate 3000 LC coupled to a QExactive orbitrap MS (Thermo Fisher Scientific, Bremen, Germany). Chromatography was performed using a Gemini column (2.1 mm x 100 mm, 3 um; Phenomenex, Torrance, CA, USA). HRAM determination was performed in full MS and data-dependent MS/MS modes. Differential and in silico dissociation software analysis was performed using SIEVE® and Mass Frontiers® packages (Thermo Fisher Scientific).

In an effort to understand the processes applied by the proprietary SIEVE® software package, individual steps of the differential analysis workflow were evaluated using conventional parameters for method assessment. Firstly, chromatographic alignment was found to be the most critical to successful identification of 'unknown' responses. Secondly, appropriate detection limits for NBOMe compounds in equine urine were achieved following optimization of parameters that improved signal to noise (S/N). Thirdly, the most robust statistical measure provided by SIEVE® was determined for translation to routine testing. Precursor Ion Fingerprinting (PIF) was applied to dissociation results provided by the Mass Frontiers® software package, however the limitation of MS2 data maintained the requirement for literature input to characterize 'unknowns' thus precluding true in silico identification in the absence of MSn.

The proposed workflow can be applied by forensic toxicology laboratories to implement fit-for-purpose non-targeted screening methods to support accreditation under ISO17025 and service delivery to customers.

FRIDAY, September 4th

11.00-12.45 pm SESSION 2 – post mortem Forensic Toxicology

FOP 9 A CLUSTER OF PARA-METHOXY-METHAMPHETAMINE (PMMA) RELATED FATALATIES

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PMMA is the para-methoxylated analogue of methamphetamine. From time to time, there have been outbreaks of PMMA use leading to death or severe side-effects. The symptoms and the cause of death from PMMA intake seem to be connected with serotonin syndrome. It is well known that serotonergic drugs should be used with caution in combination with MAO inhibitors, because of the risk of increased levels of both serotonin and other monoamines. However, PMMA is also potent MAO-A inhibitor and may produce serotonin syndrome by itself, leading to serious effects on the cardiovascular system. Common symptoms of PMMA intoxication are hyperactivity, hallucinations, muscle spasms, convulsions, hyperthermia, rhabdomyolysis, tachycardia, cardiac arrest and multiorgan failure.
Here we report seven deaths related to PMMA intake over a short period of time and within a narrow geographic area.
Routine toxicological screening in femoral blood for pharmaceuticals and drugs was performed with liquid chromatography time-of-flight mass spectrometry and positive findings were confirmed and quantified using HS-GC, GC-MS or LC-MS/MS. PMMA and its metabolite, PMA, were quantified using LC-MS/MS after protein precipitation and dilution with acidic acetonitril:ethanol (90:10).
The deaths occurred within one week, between December 25th 2014 to January 1st 2015, in the Capitol area of Sweden. The mean age of the deceased was 21 years and the victims were 5 males and 2 females. Six out of seven cases presented with severe lung edema, a typical but unspecific finding in drug related deaths. Some also showed brain edema and hypoxia. Three of the deaths were witnessed with signs of breathing difficulties prior to collapsing or seizures before going into cardiac arrest. The others were found dead at home or outdoors. All cases also presented with other drugs of abuse in femoral blood, MDMA, amphetamine, cocaine, and tetrahydrocannabinol. The concentrations of PMMA were generally high, 3.3-7.1 µg/g with one exception where the decedent was treated at hospital for a longer period (0.03 µg/g). The PMA concentrations reflected those of metabolism with 6-10% of the PMMA concentration. The circumstances, symptoms and the high concentrations of PMMA points towards acute PMMA overdose. Very quickly the media reacted and reported about deaths occurring after people had ingested pills with the "Superman" logo and this may have contributed to stopping this isolated occurrence from spreading. We conclude that PMMA is a dangerous drug that may cause accidental overdose deaths.

FOP 10 THE GAS PROJECT : POSTMORTEM IMAGING AND ANALYSIS OF CARDIAC GASES HELP TO DIAGNOSE THE CAUSE OF DEATH

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With the increase of forensic imaging tools, the monitoring of intracadaveric gases has become possible. These gases seem to be useful indicators for the forensic diagnosis.
The gas project is a multidisciplinary research project implying forensic imaging and gas analysis to advance in two directions. Firstly, in a diagnostic direction by learning about the distinction between gas due to postmortem phenomena (essentially putrefactive) and antemortem phenomena linked to the causes and circumstances of the death such as an air embolism or a diving accident for example. The second direction is concerning thanatology because the results of our study can improve our knowledge on postmortem putrefactive phenomena and human taphonomy.
We have investigated 55 cadavers to set up and to develop a standardized method for sampling and analysis of gas found in different anatomical components, and to identify reliable gas indicators among oxygen (O_2), nitrogen (N_2), hydrogen (H_2), hydrogen sulphide (H_2S), methane (CH_4) and carbon dioxide (CO_2). Gaseous sites such as cardiac region were identified firstly by Multi Dimensional Computed Tomography (MDCT) non-enhanced scans using eight-row unit (GEHealthcare). Gas syringes with needles and taps were installed under MDCT guidance for a precise sampling. Gas samples were transferred in Headspace vials, hermetically closed and previously filled with water. Gas analyses were performed by Gas Chromatography-Mass Spectrometry / Thermal Conductivity Detection. The bodies were divided in three categories : Embolism (E) - 3 cases, gas presence consecutive to traumatic injuries (T) - 4 cases and alterated cases, this latter being subdivided in three groups (Beginning Alteration - 21 cases, Moderate Alteration - 10 cases and Advanced Alteration - 2 cases).
The gas composition identified in the cardiac region of the different categories of cases has permitted to define clear indicators. A vital and massive gas embolism is diagnosed when: 20 % < CO_2 < 50 %, CO_2/N_2 > 0.2 and gas volume > 10 mL. Gas presence consecutive to traumatic injury is diagnosed with a gas volume < 10 mL, CO_2 <20 % and CO_2/N_2 <0.2. Alteration is diagnosed as soon as CH_4, H_2 or H_2S is detected. More precisely, beginning alteration is characterised by: O_2 ≤15 %, H2 <10 % and if present, H_2S,CH_4 < 1 %. Moderate alteration is characterised by: O_2 <15 %, H_2 >10 %, H_2S >1 % and CO_2/N_2 >0.2 and finally, advanced alteration is characterised by: CO_2 <25 % and CH_4 >1 %.

Thanks to the standardized protocol of MDCT-guided gas sampling and analysis,bodies can be sorted according to the presence of intracadaveric gases and their respective compositions. The distorsions between macroscopic examination and forensic imaging (case of a body without external sign of alteration but gas presence noticed by forensic imaging) are easily solved by gas analysis. Gas analysis is become a mandatory tool to investigate cases with a known history of embolism or as soon as a gas volume is detected by forensic imaging in order to strengthen the medicolegal conclusions.

FOP 11 DETECTION OF PSYCHOACTIVE SUBSTANCES ON HAIR AND MUSCLE SAMPLES AFTER 30 YEARS BY LC-MS/MS AND MALDI-LTQ ORBITRAP TECHNOLOGY WITH DRIED MATRIX SPOT SAMPLING

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A body of a 43 years old man was found recently together with other 26 bodies near a cemetery just 1.20 m from the surface wrapped in a plastic sheet. The man could be identified through DNA analysis and dental recognition; he died in 1984 probably by suicide but the cause of death remains unknown. The only available samples, hair and muscle, were taken at autopsy and analyzed by LC-MS/MS and MALDI LTQ Orbitrap technique with dried matrix spot sampling. The aim of the present study was to verify the possibility to detect psychoactive substances on post-mortem samples (hair and muscles) taken from a body 30 years after the death.
Soil and organic debris were gently removed from hair samples prior to the washing procedure (twice with dichloromethane). For drugs determination, pulverized hair samples (2 x 50 mg) were extracted overnight with methanol at 50°C and purified by solid phase extraction (Evolute CX, Biotage). Solutions were finally analyzed with LC-MS/MS (TSQ Vantage, Termofisher). For ethyl glucuronide (EtG) determination, 50 mg of pulverized hair sample were placed in 1.5mL water and sonicated for 1 hour. The extract was then purified by solid phase extraction (Evolute AX, Biotage) and analyzed with LC-MS/MS (Xevo TQD, Waters). The muscle sample was homogenized with deionized water (1:1); 20 ul were deposed on a GE 903 protein saver card. The spot was cut and placed in an Eppendorf 2 ml tube with 500 ul of methanol. The solution was sonicated for 15 minutes then evaporated; the residues were reconstituted with 100 ul of mobile phase and injected into the LC-MS/MS system (AB Sciex QTrap® 4500). Furthermore, 5 um slices of deep frozen muscle were analyzed by MALDI-LTQ Orbitrap technology.
Screening and quantitative analyses of hair samples allowed the detection of: amitriptyline, nortriptyline, 7-amino-flunitrazepam (<10 pg/mg), desmethylflunitrazepam (<10 pg/mg), diazepam (690 pg/mg) and temazepam (<20 pg/mg. The presence of these substances was confirmed in muscle. No ethylglucuronide was detected in hair or muscle samples.
The detection of benzodiazepines (diazepam, temazepam, 7-aminoflunitrazepam and desmethylflunitrazepam) and antidepressants (amitriptyline and nortriptyline) on 30 years old postmortem hair and muscles samples was possible by means of LC-MS/MS and MALDI LTQ Orbitrap techniques.

FOP 12 THE SEARCH FOR A VOLATILE HUMAN SPECIFIC MARKER IN THE POST MORTEM DECOMPOSITION PROCESS

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In forensic science, locating the body is one of the biggest challenges for police forces, but obviously of utmost importance for the progress of the investigation. A multidisciplinary approach is currently used: manual probing, thermal imaging, ground penetrating radars and cadaver dogs. The biggest disadvantage of these approaches is that they are not human specific, i.e. they cannot discriminate between human and animal remains. Therefore, in this study we chemically analyze and compare the human and animal decomposition process in order to find human specific marker(s). This marker can be a great tool in the more specific training of cadaver dogs and might lead to the development of a portable device that is sensitive enough to locate human bodies.
The aim of this study is to identify a human specific volatile organic compound (VOC) that is released during the decomposition process in laboratory environment. The identified compounds are used in cadaver dog tests in order to evaluate their potential use in a more specific dog training.
In this study, we sampled the headspace of 6 human and 26 animal remains (mammals, fish, amphibians, birds and a reptile) that decomposed in glass jars in a laboratory environment. Using a sorbent tube coated with Tenax®, VOCs were collected during 6 months by pumping 2 L of air at a flow of 100 mL/min. Afterwards, the sorbent tubes were desorbed and analyzed with a validated method using a thermal desorber combined with gas chromatograpy coupled to mass spectrometry (TD-GC/MS). When the VOC-profiles of human and animal remains were identified, principal component analysis (PCA) was applied on the results to search for (a) human specific marker(s). To apply our hypothesis that these substances could allow to distinguish human from animal remains, six trained cadaver dogs were used to see whether they would react to the substances as if it were human remains. A mixture of identified compounds was tested amongst other fragrances (negative controls) in an experimental set-up. The response of the canines to the mixture was observed.
In the headspace of the human and animal remains, 452 different VOCs were identified. After PCA-analyses, a mixture of 8 compounds (ethyl propionate, propyl propionate, propyl butyrate, ethyl pentanoate, pyridine, diethyl disulfide, methyl(methylthio)ethyl disulfide and 3-methylthio-1-propanol) was identified as possible human specific compounds. Nevertheless, none of these 8 separate compounds was human specific, but a combination did allow the distinction of human and pig remains from other animal remains. Four of six canines gave a positive response to the mixture. In future tests, dogs will be trained with the identified VOCs to see if they are able to find human

remains and more importantly distinguish human from animal remains.
A combination of 8 VOCs allows the distinction of human and pig remains from other animal remains in a lab-controlled decomposition study. First tests indicate that cadaver dogs recognize the human specific mixture as decomposition fragrance. Additional research on whole corpses, in-the-field measurements and more extensive testing with dogs has to corroborate these results.

FOP 13 QUETIAPINE IN BRAIN TISSUE

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Brain tissue is a valuable alternative to blood in postmortem forensic investigations, but scarcity of information on reference levels in brain tissue makes interpretation challenging. Antipsychotic drugs are of particular interest in postmortem investigations, since patients with schizophrenia have a life expectancy 10-25 years shorter than the background population. To make a reliable estimation of the cause of death in cases involving antipsychotic drugs, postmortem reference levels are important.
The aim was to estimate reference values for non-toxic and toxic levels of the antipsychotic drug quetiapine in brain tissue and relate those to concentrations in postmortem blood.
Quetiapine was quantified in blood and brain tissue in 43 cases. Quantification in blood was performed by liquid-liquid extraction and UHPLC-MS/MS, while brain concentrations were measured by solid phase extraction and UHPLC-MS/MS. The results were pooled in groups according to the cause of death: One group, where quetiapine was unrelated to the cause of death, and two groups, where quetiapine had been ruled as a contributing or the only cause of death, respectively.
For cases where quetiapine was unrelated to the cause of death (N=36) the 10-90 percentiles for quetiapine concentrations in brain tissue were 0.03 - 1.54 mg/kg (mean 0.79 mg/kg, median 0.48 mg/kg). Corresponding blood 10-90 percentile values were 0.01 - 0.39 mg/kg (mean 0.19, median 0.15), giving brain-blood ratio 10-90 percentiles of 2.31 - 6.54 (mean 4.32, median 3.87). Thus, on average the brain concentrations were about four times the blood concentrations. For cases where quetiapine was a contributing cause of death (N=5) the median value in brain tissue of 8.03 mg/kg (range 2.69 - 22.98 mg/kg) was more than 15 times higher than the median of the non-toxic values, and about the same relationship occurred for blood with a median of 3.19 mg/kg (range 1.01 - 6.91 mg/kg). The brain-blood ratios for toxic levels were in the range 2.08 - 6.05, which correspond to those of the non-toxic levels. A single case, where quetiapine was ruled as the sole cause of death - a suicide by quetiapine overdose - had an even higher value of 25.74 mg/kg in brain tissue. The blood concentration was 8.99 mg/kg, giving a brain-blood ratio of 2.86.
The brain concentrations of quetiapine observed in cases, where quetiapine was judged not to be related to death, may serve as a reference, when evaluating postmortem cases where blood is unavailable. The recorded concentrations, where quetiapine was judged as a contributing cause of death, give an indication of likely toxic levels. Both blood and brain values correspond well to the limited amount of data found in the literature, and the brain-blood ratio was stable and unrelated to cause of death.

FOP 14 AUTOPSY AND TOXICOLOGY FINDINGS IN THREE CASES OF INTRAVENOUS INJECTION OF ORAL TABLETS

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The practice of crushing pills, dissolving the powder in water and injecting the material intravenously is dangerous and potentially lethal. Potential mechanisms of sudden death in such cases include overdose of medication, anaphylaxis and acute right heart failure due to secondary pulmonary hypertension (cor pulmonale).
The aim of this presentation is to report the autopsy and post mortem toxicology findings in three unrelated cases of death attributed to the practice of injecting solutions of crushed pills.
Cases of sudden unexpected death including those deaths attributable to drug use or misuse are by statute reported to the Tasmanian Coroner. Two of these three persons were hospitalised prior to death; in those cases ante-mortem hospital acquired venipuncture blood was available for toxicology testing in addition to detailed clinical observations. Each of the deaths was witnessed by other persons. Complete forensic autopsy including histology of organs was performed in each of the cases during which samples were collected for toxicology testing.
Each of these cases represents a sudden death caused as a direct result of intravenous drug use. None of the deaths were the result of acute overdose of medication as indicated by drug concentrations reported. Each of the deaths was witnessed including two who were hospitalized prior to death. All of the individuals developed marked respiratory and or cardiac failure immediately prior to death. At autopsy, all had marked pathology of the lungs characterised by florid foreign body granulomas and copious foreign material (e.g. microcrystalline cellulose used as a binder in tablets intended for oral ingestion) that was deposited within the capillaries of the lungs. The presence of foreign body granulomas within the lungs indicated previous episodes of injection of foreign material. With each successive episode of injection, additional material deposited in the pulmonary capillaries caused increased resistance of pulmonary blood flow (secondary pulmonary hypertension). During the final, fatal episodes of intravenous injection new deposits and further increased resistance to pulmonary blood flow exceeded the capacity of the heart to compensate resulting in death by acute right sided heart failure (cor pulmonale).
Toxicology data used in the course of death investigation is optimally interpreted in the context of a complete autopsy. The toxicology data in these cases supported the history that specific drugs were injected (alprazolam and methylphenidate) however toxicology data alone would fail to demonstrate a cause of death.

FOP 15 FATAL COMBINATION WITH 3-METHYLMETHCATHINONE (3-MMC) AND GAMMA-HYDROXYBUTYRIC ACID (GHB)

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Over the last decade, new psychoactive substances (NPS) have rapidly emerged in the drug market. There are designed to cause similar effects to controlled drugs and are considered as "legal" alternatives for some of them. 3-methylmethcathinone (3-MMC) is a designer drug from the substituted cathinone family with a structure close to mephedrone (4-MMC), not yet classified as a drug of abuse in France. Despite increasing use of such compounds in France, only few fatalities have been described.

We reported the case of a 69-year old man who was discovered dead. 3-MMC and "poppers" (alkyl nitrites) were found at the scene by the police. Autopsy samples (peripheral and cardiac blood, urine, gastric content, bile and hair) were sent to our laboratory to document a possible death involving abuse of substances.

Routine toxicological analysis was performed with GC-FID, HPLC-DAD, HS-GC-MS, GC-MS, LC-MS-MS and LC-QTOF. GHB was analyzed by GC-MS for fluids and GC-MS-MS for hair. After liquid-liquid extraction with alkaline pH, 3-MMC was identified with GC-MS (that allows the discrimination with 4-MMC) and quantified with UPLC-MS-MS with the 2 following transitions: 178.1 > 160 and 178.1 > 144.9.

Toxicological analysis of peripheral blood revealed the presence of 3-MMC (0.33 mg/L), pseudoephedrine (0.03 mg/L) and GHB (576 mg/L). These molecules have also been found in other post-mortem fluids. Using LC-QTOF, hydroxylated metabolite of MMC was identified in urine. Furthermore, test of "poppers" by HS-GC-MS was negative. Hair analysis, without segmentation, demonstrated the presence of 3-MMC (206.7 ng/mg), pseudoephedrine (0.16 ng/mg) and GHB (96.3 ng/mg) and suggested a repeated consumption of these substances. However, one cannot exclude contamination by sweat during the agony. The presence of GHB can be explained to control myoclonia and excessive sudation due to cathinone derivatives.

Toxicological postmortem results suggest a fatal combination of 3-MMC and GHB. It is surprising that a 69-year old man was involved in such a case, but he was known to participate in the night-abusing population. This case illustrated the use of NPS and synthetic products and the easy possibility to obtain them via open access on Internet.

FOP 16 TIME-DEPENDENT POSTMORTEM REDISTRIBUTION OF CENTRALLY ACTING SUBSTANCES

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Often several hours pass by between the death of a person and the autopsy, where samples for a toxicological analysis can be collected. However, it is well known that substance concentrations can change significantly during these hours. The phenomenon of postmortem redistribution (PMR) is a well-known problem in forensic toxicology and complicates interpretation of postmortem concentrations considerably.

The aim was to investigate PMR for centrally acting substances from various drug classes in various matrices by comparing the substance concentrations in human body fluid and tissue samples collected at different time points after death.

Cases with suspected substance intake shortly before death were selected for the study. After the routine computer tomography (CT) scan, biopsies (ca. 20 mg) were taken in triplicates from liver l. dexter, kidney, lung, spleen, muscle and adipose tissue (AT) and 0.5 ml femoral blood, heart blood and cerebrospinal fluid (CSF) were collected. The exact position of the sampling area was planned and controlled by a CT coupled to a robotic arm carrying a biopsy tool (Virtobot®). At autopsy, samples from the same body regions were collected manually. A urine sample was collected only at autopsy. Centrally acting substances were quantified in all collected matrices using a validated LC-MS/MS-method for 83 analytes from different drug classes, e.g. antidepressants, antipsychotics, benzodiazepines, opioids (Staeheli, Kraemer, Steuer, GTFCh, Mosbach, 2015) and the concentration changes between the time points and the distribution between the matrices were investigated using Student`s t-test ($p<0.05$).

Up to now, results from 10 cases including 22 substances (opiates, opioids, antidepressants, antipsychotics, benzodiazepines) have been included in the study. Postmortem time intervals were between 6 and 47 hours. Most of the substances showed highest concentrations in liver, lung, kidney or spleen. Lowest concentrations were typically found in CSF or AT, often followed by blood and muscle. PMR indicated by concentration changes higher than the uncertainty of measurement of the method was observed in all investigated matrices. So far, lowest PMR was observed for bromazepam and oxazepam. A decrease (not significant) in FB concentrations was observed for e.g. lorazepam (up to -35 %), diazepam (-20 %) or hydroxyrisperidone (-10 %). Significant increase in FB concentrations was observed for clomipramine (+95 %), trimipramine (+65 %) or venlafaxine (+30 %). Differences could add up to > 100 %, with the extent of PMR in the majority of cases being more dependent on the analyte rather than on the matrix.

PMR leading to significant changes in substance concentrations could be observed in all investigated matrices and seems to be substance specific rather than matrix dependent. Even if more samples have to be analyzed for deeper insights, it is obvious, that interpretation of postmortem concentrations still has to be done with great caution.

Poster abstracts

P1 – P137 SESSION I and II

P1. HIGH SENSITIVITY ANALYSIS OF OPIOIDS IN ORAL FLUID USING IONKEY/MS

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Over the past three decades oral fluid has emerged as a highly valuable biological specimen and is now commonly used in numerous settings including therapeutic drug monitoring and for drug testing within the workplace and at roadside. A key analytical challenge surrounding oral fluid analysis is the capability of analyzing small volumes using high sensitivity instrumentation that is robust and easy to use.
Microfluidic technology offers several advantages for toxicology and forensic laboratories. The combination of ease of use and reduced sample solvent consumption offers reduced operating costs both on operator time and solvent consumption. Clamp-on microfluidic fittings allow facile replacement of the microfluidic device in seconds, facilitating method development with different column chemistries. Operating on the micro-scale, 2-6 µL/min flows, leads to significant improvements in sensitivity for many analytes when compared to conventional 2.1 mm diameter Acquity UPLC column formats.
To investigate the utilization of ionKey/MS for the high sensitivity and robust analysis of opioids in oral fluid matrix for forensic applications.
Blank oral fluid was collected from volunteers using QuantisalTM collection devices (Immunalysis). Following collection oral fluid was spiked with a mixture of 25 opioids to give a range of concentrations (0.1 to 350 ng/mL) and vortex-mixed. Five microlitres of the spiked oral fluid was injected onto the analytical system which comprised a Waters NanoAcquity UPLC M-Class coupled to a Waters Xevo TQ-S Mass Spectrometer. The system utilized a trapping valve manager fitted with a trapping column (300 µm id x 50mm length, T3 5.0 µm 130Å); analytical separation of the opioids was achieved through use of an iKey UPLC T3 1.7µm 130Å analytical tile. Data quality for the opioids was assessed using a MS method comprising 25 MRM transitions.
Performing analysis of oral fluid samples taken directly from an oral fluid collection device provided a streamlined method for reducing workflow and increasing throughput. The use of a trapping column prior to analytical separation allows the removal of any additives e.g., surfactants that can cause suppression in LC/MS and lead to reduced sensitivity (observed matrix effects of up to 95% for a panel of opioids). Utilization of the ionKey/MS system enabled a 3-47X improvement in sensitivity compared to an ACQUITY 2.1 mm ID column when injecting the same volume. In addition to morphine, many of the glucuronides were also identified as having improved sensitivities. Specifically, morphine-3b glucuronide was demonstrated to be baseline separated from morphine, with a sensitivity improvement of 11X; whilst this particular glucuronide may not be relevant for oral fluid, the increased sensitivity presents a clear advantage for analysis in other biological specimens. Recoveries of the opioids were analyte dependent and ranged from 59-100% as compared with the direct inject mode. The assay described here is robust and reproducible using oral fluid matrix injections of over 500 injections. Retention time reproducibilities were < 1% RSD, while peak area reproducibility of morphine was 11.1% RSD.
We demonstrate a high sensitivity analytical tool that is robust enough for high throughput analytical forensic laboratories.

P2. ANALYTICAL STRATEGY FOR THE DETECTION OF SUBITRAMINE IN DIETARY SUPPLEMENT BY 6550 IFUNNEL Q-TOF LC-MS

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Adulteration of botanical food supplements with undeclared synthetic drugs is becoming a widespread and mostly uncontrolled problem in many countries. Among them, slimming functional food are commercially readily available to a vast unaware population. At the moment, there are no established analytical protocols for the systematic detection of synthetic adulterants in these products but a large body of literature is converging to the target screening approach, either by liquid chromatography or gas chromatography. However, this approach may not be suitable due to the sheer number of chemicals. For this reason, high-resolution high-accuracy mass spectrometry (HRMS), enabling accurate-mass determination of ionic species (and metabolites), offers the potential to overcome the limitations of multi-target screening.
The present work shows a simple but effective approach to detect sibutramine and caffeine in allegedly 'natural' herbal extracts by Q-TOF LC/MS technology.
Samples were suspended in water and ultrasonicated for 10 minutes. For the screening, 50 µL of sample was diluted in 150 µL of water and directly injected in LC-Q-TOF. For confirmation, a second aliquot of 1 mL of each sample was liquid-liquid extracted. Separations were carried out in an Agilent ZORBAX Eclipse Plus C18 column, 2.1 X150mm, 1.8µm at constant flow 0.2 ml/min. Mobile phase A consisted of water added of 0.01% formic acid and phase B of methanol added of 0.01%. Toxicological screening was based on a combination of full scan accurate-mass and accurate-mass fragmentation pattern followed by library match through a dedicated MS/MS spectral library. For confirmation, Target MS/MS analysis on samples and pure reference standards was performed.
Compound identification was obtained by matching accurate mass, retention time (if available) and CID fragmentation patterns data. In this way, caffeine and sibutramine were confirmed and quantified. The limits of detection (LOD), defined as S/N=5 were 0.5 and 2 ng/ml for sibutramine and caffeine respectively; limits of quantification

(LOQ), defined as S/N= 10 were 1 ng/ml for sibutramine and 4 ng/ml for caffeine. Sibutramine was found in two out of the three analyzed samples at a concentration of 0.6 and 1.04 µg/mg. The caffeine content varied from 5 to 100 mg per sample/pack.
In this application, the applicability of accurate mass measurements (Q-TOF LC/MS) in the investigation of undeclared active compounds in commercial foodstuffs was demonstrated in an easy and rapid method set-up.

P3. SYNTHESIS OF THE "KROKODIL" DRUG THROUGH "STREET" REPORTED METHODS, AND PRODUCT ANALYSIS

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"Krokodil" is the street name attributed to a homemade drug of abuse originated on Russia and Ukraine. which is attracting the researchers' attention due to its extreme toxicity, characterized by skin necrosis, giving the rotten flesh aspect on addicts, and short lifespan. Used as a cheap alternative to heroin, "krokodil" is synthesized from codeine-containing tablets, using raw materials, such as iodine and phosphorus, which are easing accessible on any drugstore. With these materials, addicts perform a chain of chemical reactions, converting codeine into desomorphine (5a-17-methyl-4,5-epoxymorphinan-3-ol), a semi-synthetic opiate derivative, which is claimed to be the substance responsible for the addictive effects.
In this work, we followed a protocol for the synthesis of "krokodil" based on the reports obtained from users, aiming to understand chemistry behind krokodil production, and analyze the products formed.
The starting materials for "krokodil" synthesis are tablets containing codeine, caustic soda, gasoline, hydrochloric acid, iodine from disinfectants and red phosphorus from matchboxes, all of which are easily available in a retail market or drugstores. The manufacture of "krokodil" involves two steps. Firstly, a simple acid-base extraction of codeine from the tablets, using gasoline as organic solvent is performed. The second step is the reduction reaction of codeine to desomorphine, using iodine and red phosphorus. The resulting mixture is light brown liquid and has a strong acidic pH that is injected without previous purification. In order to assess the chemical profile of the synthesis products, analytical gas chromatography-electron impact/mass spectrometry (GC-EI/MS), thin-layer chromatography (TLC) and High Performance Liquid Chromatography (HPLC) were performed. A GC-EI/MS methodology was used for detection and quantification of desomorphine and codeine.
The methodology for detection and quantification of desomorphine and codeine was developed and validated. This method proved to be selective, sensitive and robust, and the regression analysis for both analytes was shown to be linear in the range of 0.62-10.00 µg/mL, with correlation coefficients of 0.9982 and 0.9995 for desomorphine and codeine, respectively. The coefficients of variation did not exceed 15%. The LOD were 0.15 and 0.17 µg/mL, and the LOQ were 0.49 and 0.57 µg/mL for desomorphine and codeine, respectively.The quantitative analysis of the product obtained by our procedure revealed the absence of codeine and the presence of desomorphine, which is the "krokodil" marker. The concentrations of desomorphine found were about 1.5 mg per dose, which is capable to cause euphoria and analgesia in addicts. The qualitative analysis of the synthesis products showed the presence of organic impurities originated from procedure and other two morphinans (dihydromorphine-3,6-dideoxy and morphinan-4,5- epoxy-3-ol). The presence of these morphinans are in accordance with street samples previously analyzed.
In this work we have successfully performed the "street" synthesis of "krokodil" and analyzed the products formed, confirming the full transformation of codeine, and the formation of desomorphine but also of other morphinan derivatives. This allows us to infer that the psychotropic effect of "krokodil" may be derived from an amalgam of morphinans besides desomorphine.

P4. DEVELOPMENT OF AN UHPLC-MS/MS METHOD FOR DETERMINING GHB AND GHB GLUCURONIDE CONCENTRATIONS IN HAIR AND APPLICATION ON FORENSIC CASES

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GHB (gamma-hydroxybutyric acid) is a low-molecular-weight substance with two polar substituent groups (hydroxyl and carboxyl) naturally present within mammal species. It is a drug of abuse ("liquid ecstasy", "fantasy") and is known as a date-rape drug due to its ability to induce drowsiness, forgetfulness and muscle relaxation. GHB may disappear rapidly due to its short half-life and generally it may be difficult to distinguish between endogenous levels and levels related to ingestion of GHB. Thus, determination of GHB glucuronide as a metabolite of GHB might be of relevance. Measurement of GHB glucuronide in other matrixes than urine has hitherto not been performed. Therefore, we were interested in measuring the glucuronide in hair.
The aim of the present study was to develop and validate a sensitive UHPLC-MS/MS method for simultaneous determination of GHB and GHB glucuronide in human hair and then apply the validated method on a routine basis for forensic toxicology cases.
Several sample preparation procedures were examined. In the optimized method the analytes were extracted from 10 mg of hair by incubation in 1½ h in a 25:25:50 (v/v/v) mixture of methanol: acetonitrile: 2 mM ammonium formate (8% acetonitrile, pH 5.3). Subsequently, GHB and GHB glucuronide were determined by UHPLC-MS/MS. Negative ion electrospray ionization and MRM mode were applied.

Poster abstracts

The calibration curve was from 0.32 and 0.48 ng/mg to 50 ng/mg hair for GHB and the glucuronide, respectively. Extraction recoveries were from 62 to 92% for the analytes, and the accuracy was from 90 to 108%. Precision based on daily controls were from 9.1 to 11.3% for GHB and 12.2–17.9% for GHB glucuronide. Endogenous levels were up to 1.0 ng/mg for GHB and 1.2 ng/mg for GHB glucuronide in control subjects. In two GHB abusers, GHB was highly elevated (461–591 ng/mg), whereas GHB glucuronide was only slightly elevated in one of the subjects (1.7–3.1 ng/mg) and below the LOQ in the other. The GHB concentration in the abusers was much higher than in previously published reports. The samples were reanalyzed in a smaller fraction to adjust to the calibration curve.
A satisfactory method was developed to determine GHB and its glucuronide in hair. Thus, apparently GHB glucuronide is not incorporated in hair in any appreciable amounts in GHB addicts and does not contribute diagnostically with regard to detection of exogenous exposure.

P5. DETERMINATION OF NEW PYRROLIDINO CATHINONE DERIVATIVES, PVT, 4F-PVP, MPHP, PV8, PV9 AND 4F-PV9, IN HUMAN BLOOD BY MALDI-Q-TOF MASS SPECTROMETRY

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Recently synthetic cathinone derivatives have widely been distributed as drugs of abuse in the world, and their poisoning cases were often encountered. In such cases a rapid and sensitive analysis is highly demanded. As a high throughput and sensitive method, matrix-assisted laser desorption ionization (MALDI) quadrupole time-of-flight mass spectrometry (Q-TOF-MS) was remarked, but its application to the analysis of small molecules was so far limited.
The aim of the present study is the validation of a MALDI-Q-TOF-MS method for the simultaneous quantitation of pyrrolidino cathinone derivatives such as PVT, 4F-PVP, MPHP, PV8, PV9, 4F-PV9 and the identification of their metabolites using only 20 µl of blood.
Twenty microliter of blood and 60 µl of 0.13 M potassium carbonate were mixed and centrifuged at 10,000 g for 2 min. The supernatant (72 µl) was placed in a new tube and 220 µl of 1-chlorobutane (CB) was added. They were vortex-mixed for 1 min and centrifuged at 10,000 g for 2 min. The upper 198 µl of the CB layer was placed in another tube and evaporated to dryness. The residue was mixed with 16 µl of α-cyano-4-hydroxy cinnamic acid solution. Two µl of the solution was loaded on a sample plate and allowed to dry. A reflector type Q-TOF mass spectrometer, QSTAR Elite Hybrid (AB SCIEX) with mass resolution of 15,000 was used in the positive ion mode. Mass spectra were obtained using 355-nm radiation from Nd:YAG laser; the excitation energy, 3.4 µJ; the delayed extraction voltage, 20 V; 20 cycles at 100 Hz.
MS spectra of six cathinones showed protonated molecule [M + H] and iminium, respectively. For their quantitation, α-PVP was used as the internal standard. The limit of detection was 1 ng/ml and the quantitation range was 2 – 100 ng/ml for six cathinones, respectively. We encountered a fatal poisoning case where PV9 was abused. The PV9 levels in postmortem blood samples in right heart, left heart and femoral vein were 198, 209 and 163 ng/ml, respectively. Three metabolites of PV9 were detected. The peak of the oxidized metabolite was highest among those of metabolites and [M+H]. The differences between the observed mass and calculated mass of three metabolites of PV9 were less than 0.0007 Da. The fragment ions obtained by MS/MS suggested that the oxidation of PV9 occurred mainly on its alkyl chain.
The sensitive detection of cathinone derivatives by MALDI-Q-TOF-MS using the extract from 20 µl of blood was performed within 30 s per sample. The method is also suitable for the identification of metabolites because it provides precise molecular masses and MS/MS data to identify metabolites without their standard samples.

P6. SIGNIFICANT INCREASE IN THE SCREENING CAPACITY OF DRUGS OF ABUSE IN ORAL FLUID THROUGH THE USE OF BIOCHIP ARRAYS

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Laboratory tests of oral fluid have continued to expand as it represents an excellent alternative matrix for monitoring drug intake in treatment, workplace and driving under the influence of drugs (DUID). The collection of oral fluid is simple and non-invasive and can be easily observed. Immunoassays are highly selective antibody-based tests that provide high throughput screening of a range of drugs and their metabolites in different matrices. Biochip array technology has been used to provide multiplex screening of different drug classes from a single sample. This is relevant when the volume of sample available for analysis is limited.
The aim of this study was to expand the screening capacity of drugs of abuse in oral fluid even further through the use of two biochip arrays for the multiplex screening of 23 classes of drugs of abuse at a selected cut-off concentration relevant for an oral fluid testing laboratory.
Competitive chemiluminescent immunoassays were employed. The capture antibodies are immobilised and stabilised on the biochip surface defining microarrays of discrete test sites. The immunoassays were applied to the Evidence analyser. Two biochips were used for the semi- quantitative detection of 23 drug classes including: amphetamines, barbiturates, benzodiazepines, methadone, opiates, PCP, cocaine, oxycodone, propoxyphene, cannabinoids, fentanyl, buprenorphine, tramadol, meprobamate, synthetic cannabinoids, dextromethorphan, tricyclic antidepressants, meperidine, methylphenidate, mitragynine and ketamine. For each biochip 25 µl sample volume was used. Results for up to 90 oral fluid samples were generated for all 23 drug classes within 120 minutes. Oral fluid was collected with the Quantisal collection device following manufacturer's instructions.

For all drugs tested the calculated limit of detection (LOD) was below the selected cut-off i.e. methamphetamine 0.59ng/mL (cut-off:20ng/mL), benzodiazepines 0.18ng/mL (cut-off:10ng/mL), fentanyl 0.07ng/mL (cut-off:1ng/mL), buprenorphine 0.05ng/ml (cut-off:1ng/mL). The LOD was determined by assessing 20 negative sample replicates and was calculated as the mean +3 standard deviations. The % agreement with LC/MS was assessed for five drugs of abuse in authentic oral fluid samples and it was found to be 100% for buprenorphine (n=18), cocaine (n=33) and oxycodone (n=18), 94.4% for tramadol (n=18) and 91.7% for cannabinoids (n=60). In addition, 9 samples containing all 23 drugs spiked below, at and above the required cut-off were assessed and all samples were correctly classified as positive and negative.
This study indicates applicability of biochip array technology to increase the screening capacity of drugs of abuse in oral fluid. Two biochip arrays utilising 25 µl sample volume each allowed the simultaneous detection of 23 classes of drugs of abuse present in oral fluid samples, with results generated in 120 minutes for up to 90 samples. This represents a useful analytical tool in the screening stage of the testing process.

P7. SIMULTANEOUS DETECTION AND QUANTIFICATION OF 15 DRUGS OF ABUSE AND METABOLITES IN HAIR BY ONLINE SOLID-PHASE EXTRACTION AND LC-MS/MS

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Drugs of abuse are routinely assayed in hair in a lot of laboratories. Methods generally involve several time-consuming steps: decontamination, incubation, extraction, chromatographic assay.
A simple sample pre-treatment and online solid-phase extraction was developed to quantitate 15 drugs of abuse (amphetamine, methamphetamine, MDMA, MDA, MDEA, MBDB, mephedrone, 6-MAM, morphine, codeine, dihydrocodeine, ethylmorphine, cocaine, BEG and cocaethylene) in hair by LC-MS/MS.
Hair samples were decontaminated in successive baths: 2 x 2 min in H_2O, and 2 x 1 min in dichloromethane. After drying, hairs were cut in small pieces. 50 mg of hair were weighted and incubated overnight in methanol with internal standards, at 40°C, with agitation. The methanol was dried under nitrogen, and then reconstituted with 100 µL of 2 mM ammonium formate buffer/MeOH 70/30 (v/v). After centrifugation, 20 µL were injected in the chromatographic system. First, analytes were loaded for 1 min on the extraction column (Strata-X, Phenomenex) with a 2 mM ammonium formate buffer. Then, they were eluted on the analytical column (Kinetex PFP, Phenomenex) using a gradient with an aqueous mobile phase A (0.2% formic acid/2 mM ammonium formate), and an organic phase B (0.2% formic acid/2 mM ammonium formate in methanol/acetonitrile 70/30 v/v). The mass spectrometer was an API 3200 QTrap® (AB Sciex). The method was developed in scheduled multiple reaction monitoring (MRM) mode, with two transitions per compound. The total run time was 15 min. 14 deuterated analogues were used as internal standards for the quantification. The calibration curve (up to 10 ng/mg) was realized by spiking drug free hairs with methanolic solutions containing the compounds to be assayed. The method was validated according to the French Accreditation Committee (COFRAC) document SH GTA 04 (repeatability, reproducibility, linearity, limit of quantification and carry over). Recovery and matrix effects were measured.
Coefficients of variation (CV) and inaccuracy of the repeatability and reproducibility assays were < 15 % for all compounds. The method was linear, with a correlation coefficient $r^2 > 0,998$. The limit of quantification was 0.05 ng/mg for all analytes (CV and inaccuracy < 20%). The recovery of the incubation step in methanol was between 57 and 69 %. No carry-over was highlighted. Two compounds (MDA, MDA) showed matrix effects: five different drug free hairs spiked at the same concentration showed a CV > 15%. An external quality control allowed to verify the accuracy of the method for 9 compounds (amphetamine, methamphetamine, MDMA, MDA, MDEA, cocaine, BEG, morphine and 6-MAM).
This new analytical method was fully validated for 13 of the 15 compounds (because of matrix effects for MDA and MDEA). It can be used routinely in the laboratory to search drugs of abuse in hair samples and identify consumption weeks or months later, either for in vivo or post-mortem subjects.

P8. MULTIPARAMETER INVESTIGATION OF NEW PSYCHOACTIVE SUBSTANCES (NPS) BASED ON THE MATERIAL ORIGINATING FROM FORENSIC TOXICOLOGICAL PRACTICE AND FOCUSED ON METHODOLOGICAL AND MEDICO-LEGAL ASPECTS.

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The experience of the last decade in the field of toxicological medico-legal practice has allowed for a comprehensive view of the problem of new psychoactive substances (NPS). Most studies concern methodological problems NPS determination and their identification in preparation used. Meanwhile, the effects of drug used represent an extremely important issue of cognitive and practical. In case of NPS, the casual relationships underlying the relations between a toxic substance and its effect are complex and poorly understood, hence, there is a pressing need for investigations in this field.
The main subject of the study was determination of NPS in blood taken from living and dead subjects coming from selected cases developed for medico-legal purposes. The cases included examples of interaction effects on mental health of individual resulted in driving impairment, murder, suicide, fatal poisoning. These cases show impressively the irremediable consequences resulting from the use of NPS.
Determination of NPS in biological samples were carried out by means of LC-ESI-MS-MS and GC-EI-MS-MS following isolation procedure from biological matrix. Blood samples were subjected to solid phase extraction (SPE) using

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C18-RP columns. Prior to the GC-MS analysis, the extracts were subjected to derivatization. Determinations of NPS using the internal standards of bk-MDEA-d5, 4-MMC-d3, α-PVP-d8, MDPV-d8, JWH-073-d9, UR-144-d5 were done by the LC-ESI-MS-MS and GC-EI-MS-MS method in the MRM mode.
Presented material focused on analysis of selected examples of cases involving users of synthetic cannabinoids and cathinones representing four categories of consequences: 1) Traffic road accident - 1 case in which driver and 3 victims (2 living ones with large injury and 1 died on the spot) were under influence of cathinone - α-PVP. 2) Murders exemplified by 2 cases in which 2 perpetrators were under influence of synthetic cannabinoid AM-2201 (he killed the victim with a knife) and cathinone bk-MBDB (he killed the victim with a hammer). 3) Suicides committed by 2 man being under the influence of synthetic cannabinoid - UR-144, found as an indirect cause of death in hanging and jumping from a height. 4) Fatal poisonings with bk-MBDB, 3-MMC, 4-MMC, 4-MEC, pentedron being in character accidental and suicidal.
Consequences of using novel psychoactive substances through illustrating such effects with cases originating from the medico-legal practice, demonstrate via multiparameter documentation the wide range of consequences that may be derived from an individual reaching for an unknown substance in order to experience altered states of consciousness. The medico-legal practice shows that experiments with narcotic novelties embarked on by young people may turn the lives of such individuals and their families into a nightmare, making criminals of the users, ruining their health and emotional life and even leading to death.

P9. GENDER DIFFERENCES IN DRUG ABUSE IN THE FORENSIC TOXICOLOGICAL APPROACH.

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Gender differences in substance use/abuse have been the focus of research in the last 15 years. Initiation, use patterns, acceleration of disease course, and help-seeking patterns are known to be influenced by gender differences in regards to biologic, psychological, cultural and socioeconomic factors.
The paper presents a systematic review of published data on gender differences towards use/abuse of psychoactive and psychotic drugs, focusing on the importance of a multidisciplinary approach.
Basis for this paper was obtained by Medline searches using the key words "human" and "gender", combined with individual drug names or "drug abuse". The reference lists of these papers were further checked for other relevant studies.
Traditionally, drug abuse has been considered as a problem specific to men and most research has focused on the study of such gender. Actually, the gender difference is evident in adults more than in adolescents (13-19 years): adult men are 2-3 times more likely than women to develop drug abuse/dependence disorders and about 4 times as likely to have an alcohol use disorder. Such prevalence rates are not observed in adolescents, since percentages of alcoholics and drug users/abusers, as well as the gender differences disappears. Differences between men and women involve i)the biological response to drug; ii)the course or progression to dependence, iii)the comorbid psychiatric diagnoses, and may be due to both sociocultural factors and to innate biological differences. A crucial role played by ovarian hormones (estrogens and progesterone has been evidenced by both human and animal model studies.
Epidemiological data on how psychobiological and physiological peculiarities on the female gender influence vulnerability to both drug addiction and toxicological consequences of drugs taking are still in their infancy. There are still significant gaps in our knowledge mainly attributable to the lack of empirical data that only a systematic and multidisciplinary approach to the topic can generate. The introduction of the gender variable into forensic toxicological evaluations may be beneficial since it could help in the elucidation of the relationship between absorption of drugs of abuse (alone or in combination) and the onset of intoxications both lethal or non.

P10. ON-SITE SCREENING OF NEW PSYCHOACTIVE SUBSTANCES BY PORTABLE MASS SPECTROMETER

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We previously developed an 11 kg- portable mass spectrometer for rapid screening of illicit drugs, such as amphetamines, cannabis, and cocaine.
In this study, we tested capabilities of this system to detect new psychoactive substances. The system consists of a pinch valve to introduce sample gas discontinuously, a low-pressure dielectric barrier discharge ionization (LP-DBDI) source, and a miniature linear ion trap. To minimize vacuum pumps, the sample gas is introduced to the mass analyzer in only short period (20-30 milliseconds per one second) through the pinch valve. The LP-DBDI source can ionize the sample molecules as $[M+H]^+$. The system can separate the target ions from the other chemical noises without GC separation by applying tandem mass spectrometry (MS/MS).
Each methanol solution of cathinones (50 ug/mL, 1-5 uL) or synthetic cannabinoids (1 mg/mL, 1-5 uL) was attached on the top of the rod-like sampling probe and dried. After the probe was set on a heater, it was gradually heated up to 160 degrees Celsius. Vaporized samples were introduced into the LP-DBDI source and the ions of the sample were sent to the miniature linear ion trap.
We obtained mass and tandem mass spectra of 50 cathinones and 80 synthetic cannabinoids. Each result showed characteristic ions on its mass and tandem mass spectra, so that the system could identify the compounds except

for regioisomers such as 2-fluoromethcathinone and 4-fluoromethcathinone, because they had the same $[M+H]^+$ ions and the same fragment ions. We input these mass and tandem mass spectra obtained from 10 kinds of authentic standard cathinones into database of the system, and measured samples of herbal product including those cathinones. All the cathinones were automatically detected correctly within 5 minutes.
The developed 11 kg- portable mass spectrometer is possibly used on-site screening for new psychoactive substances. Acknowledgement. This work was partially supported by "R&D Program for Implementation of Anti-Crime and Anti-Terrorism Technologies for a Safe and Secure Society", Strategic Funds for the Promotion of Science and Technology of the Ministry of Education, Culture, Sports, Science and Technology, the Japanese Government.

P11. REGIOISOMERIC DIFFERENTIATION OF 2-, 3-, AND 4-METHYL-ALPHA-PYRROLIDINOBUTIOPHENONE BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY USING BROMINATION

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In Japan, with cases of cathinone-type new psychoactive substances, it is very important to differentiate between controlled and uncontrolled drugs. This can be accomplished by examining the position of a substituent on the benzene ring. In this study, the technique of bromination was examined in aiding with differentiation of three regioisomers.
Three cathinone-type regioisomers, 2-, 3-, and 4-methyl-α-Pyrrolidinobutiophenone (MePBP), were examined in this study.
Anhydrous ferric bromide (III) as a catalyst was placed in three 20 mL vials followed by 0.5 mg MePBP (a different isomer in each vial). Several drops of bromine (liquid at room temperature) were add into the vials and immediately sealed. The vials were then placed in an ice water bath (0 °C) for 10 minutes to brominate the MePBP, after which the extra bromine in the vials was removed by evaporation under a nitrogen stream. The egested bromine was trapped with saturation with a sodium thiosulfate solution. A saturated sodium carbonate solution (1mL) was added, and 3 mL of n-hexane was added to the alkaline solution to extract the compounds. The extracts were concentrated by a nitrogen stream and analyzed by gas chromatography-mass spectrometry (GC-MS) using a short narrow-bore DB-5 capillary column (10 m length, 0.1 mm i.d., 0.4 µm film thickness).
The GC-MS chromatograms for brominated 2-, 3-, and 4-MePBP showed various numbers of peaks for each compound. Each peak was numbered in the order of detection on m/z 112 mass chromatogram. The chromatograms for 2- and 3-MePBP showed 4 peaks. For 4-MePBP, only 3 peaks were observed. Thus, it was easy to differentiate 4-MePBP from the others just by observing the number of peaks. In 2- and 3-MePBP, there was a difference in the size of the peaks. In 2-MePBP, the 2nd peak was larger than the 3rd. In 3-MePBP, the peaks were switched, the 3rd peak was significantly larger than the 2nd. Then bromination of each peak was determined by mass-spectrometry, in all three MePBP, the 1st peak was identified as free. In 2-, and 3-MePBP, both the 2nd and the 3rd were identified as one brominated MePBP (one bromine atom attaching to the benzene ring), the 4th peak was 2 brominated MePBP (two bromine atoms attaching to the benzene ring). In 4-MePBP, the 2nd was one brominated MePBP and the 3rd was 2 brominated MePBP. Therefore, by examining the number and size of the peaks on the chromatogram, the three kinds of positional isomers of MePBP could easily be identified.
By using bromination along with GC-MS, the three kinds of positional isomers of MePBP could be easily distinguished by observing the number of peaks and the peak intensities that were generated.

P12. DETECTION OF THE CANNABIS METABOLITE THC-COOH IN URINE AND SERUM SAMPLES BY IMMUNOASSAY: HEIA(TM) VERSUS CEDIA®

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The detection of the cannabis metabolite THC-COOH by immunoassay is a routine procedure in clinical and forensic laboratories worldwide. The most common test designs are CEDIA® by Thermo Scientific, Online by Roche, and EMIT by Siemens. Although these assays show a good performance, there are a few issues, such as unspecific cross-reactivities and a narrow undiluted measuring range. Especially in clinical cases this is a seriously limiting factor.
The new Immunalysis Cannabinoids HEIA(TM) was tested for routine usability and compared with the CEDIA® test. The studies comparing the two immunoassays (HEIA(TM) and CEDIA®) were performed simultaneously in a forensic laboratory in Salzburg, Austria, and a clinical laboratory in Geesthacht, Germany. In addition to the usual tests for reproducibility, linearity and stability, a total of 384 urine samples and 395 serum samples were analysed. Samples showing a positive screening were quantified with validated chromatographic routine methods. The Salzburg site used a GC/MS method while Geesthacht performed a LC-MS/MS as their reference method. We calculated sensitivity, specificity, as well as negative and positive predictive value (NPV, PPV) for each lab and method.
The reproducibility of repeated analyses over a 21 day period was 3.3% to 12.3% for levels across the measuring range. The recovery for the target concentration ranged from 90% to 115%. The HEIA(TM) test allows an expanded undiluted measuring range up to 200 ng/mL compared to the CEDIA® test (100 ng/mL). Comparing the two immunoassays, 372 urine specimens gave concordant results while 12 urine specimens showed discordant results (all 12 samples were positive with the CEDIA® assay, but negative with the HEIA(TM), with only 6 samples confirmed bor-

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derline positive in chromatography). The overall NPV was calculated as 93.8% (91.1% (G) and 96.7% (S)). Using serum as the specimen matrix, 386 concordant and 9 discordant results were determined (1 of which was positive with the HEIA(TM), but negative both with the CEDIA® and in confirmation. 8 samples were positive with the CEDIA®, but negative with the HEIA(TM), and, with 1 exception, also negative in confirmation). The overall NPV was calculated as 96.1% (93.5% (G) and 99.0% (S)). When looking at the HEIA(TM) Cannabinoid and the chromatographic confirmation results, an overall accuracy of >97% was observed for urine and >95% for serum matrix respectively.
Our data demonstrate that the new HEIA(TM) THC immunoassay is comparable with the CEDIA® test for urine specimens with the advantage of an extended measuring range. Reproducibility and stability of the HEIA(TM) test was within expectations. False positive and negative results have been rare in both methods. Looking at serum as a sample matrix, the HEIA(TM) assay is showing comparable performance to CEDIA®. Some additional investigations will be initiated to finally define a specific cut-off level for serum samples with the HEIA(TM) assay.

P13. DETECTION OF BUPRENORPHINE AND NORBUPRENORPHINE IN URINE AND SERUM SAMPLES BY IMMUNOASSAY: HEIA(TM) VERSUS CEDIA®

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Buprenorphine is widely used as an analgesic and in replacement therapy. It may be obtained as a single medication or in combination with naloxone. Its first-pass metabolism requires a transdermal or sublingual application. Apart from its medical use, an extensive abuse potential is reported for buprenorphine and it is easily obtainable on the illicit market. Both the need for compliance testing of buprenorphine on the one hand and its potential for abuse on the other call for accurate screening procedures. Routine tests are currently available from Thermo Scientific and Siemens. Low therapeutic and, therefore, low specimen concentrations and a missing cross-reactivity against the main metabolite (norbuprenorphine) are the main issues with existing tests.
The new Immunalysis Buprenorphine HEIA(TM) was tested for routine usability and compared with the CEDIA® test. The studies comparing the two immunoassays (HEIA(TM) and CEDIA®) were performed simultaneously in a forensic laboratory in Salzburg, Austria, and a clinical laboratory in Geesthacht, Germany. In addition to the usual tests for reproducibility, linearity and stability, a total of 321 urine samples and 211 serum samples were analysed. Following common practice in routine testing, positive samples were quantified with validated chromatographic routine methods (LC-MS/MS). For urine specimens, a cut-off of 5 ng/mL was used. For serum specimens, a cut-off of 1 ng/mL was used for HEIA(TM) and 0.01 ng/mL for CEDIA®, respectively. We calculated sensitivity and specificity, as well as negative and positive predictive value (NPV, PPV) for each lab and method.
The reproducibility of repeated analyses over a 21 day period was 2.1% to 4.8% for levels across the measuring range. The recovery for the target concentration ranged from 80% to 117%. In contrast to the CEDIA® test, the HEIA(TM) reagent has 100% cross-reactivity against the main metabolite norbuprenorphine. Comparing the two immunoassays, 317 urine specimens gave concordant results, while 4 urine specimens showed discordant results (1 of these was positive with the HEIA(TM), but negative with the CEDIA® and the other 3 positive with the CEDIA® assay, but negative with the HEIA(TM)). Comparing the two immunoassays, the overall NPV was calculated as 97.8% (98.1% (G) and 96.7% (S)). Using serum as the specimen matrix, 190 concordant and 21 discordant results were determined (all 21 were positive with the HEIA(TM), but negative with the CEDIA®). The overall NPV was calculated as 100%. All 21 discrepant results turned out positive in LC-MS/MS confirmation. When looking at the HEIA(TM) Buprenorphine and the chromatographic confirmation results, an overall accuracy of >99% was observed for urine and 100% for the serum matrix respectively.
Our data demonstrate that the new HEIA(TM) Buprenorphine immunoassay is comparable with the CEDIA® test for urine specimens, with the advantage of cross-reactivity against norbuprenorphine. Reproducibility and stability of the HEIA(TM) test was within expectations. Looking at serum as a sample matrix, the HEIA(TM) assay is showing superior performance to CEDIA®, resulting in an overall accuracy of 100% against LC-MS/MS confirmation.

P14. ANALYTICAL APPROACH FOR THE IDENTIFICATION OF NEW PSYCHOACTIVE SUBSTANCES NPS IN SEIZURES AND A SNAPSHOT OF DRUGS CONFISCATED IN ITALY IN THE PERIOD 2013-2015.

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The diffusion of NPS in the illicit drug market is a problem worldwide. According to the UNODC "2014 Global Synthetic Drugs Assessment", 348 NPS were identified for the first time between 2008 and 2013, 97 only in 2013. A challenge for laboratories involved in the analysis of drugs is the identification of new substances, often without having reference standards.
The aim of the presentation is to report the analytical approach used for the identification of NPS in unknown seizures and to describe our analytical experience on some of the NPS confiscated in the Italian territory in the period 2013-2015.
Powders or herbal materials were dissolved in (or extracted with) ethyl acetate and analyzed by single quadrupole GC/MS in scan mode, to study the mass spectra obtained by electron impact ionization. Commercial spectral libraries were used to support unknowns identification. Solutions were further analyzed by LC/HRMS in positive ESI using an Orbitrap mass spectrometer without fragmentation to study the accurate molecular mass and isotopic cluster

in order to determine the accurate masses and raw formulas. Further analyses by HRMS, in positive ESI, applying different fragmentation voltages, were performed in order to study the accurate masses of obtained fragments and to determine their structures, especially in those cases where pure standards were not available. When a certain identification of isomers was necessary, it was performed a NMR analysis.
In the period 2013-2015 were analyzed 160 seizures from substances purchased through the internet. 32 seizures (20%) were crystals of 3-methylmetcathinone (3-MMC). This substance is controlled in Italy, but not in other Countries such as the Nederlands, from which the shipments originated. 30 seizures (19%) were crystals of 4-methylethcathinone (4-MEC), 19 confiscations (12%) were powders containing methylenedioxypyrovalerone (MDPV). N,N-diallyl-5-methoxytryptamine (5-MeO-DALT) was identified in 5 powders, ethylphenidate in five powders, pyrrolidinophenones in ten seized powders (4 α-PVP (alpha-pirrolidinovalerophenone), 4 α-PHP (alpha-pyrrolidinohexaphenone) and 2 α-PVT (α-Pyrrolidinopentiothiophenone)). Other substances identified were cathinones such as pentedrone, methylone, clephedrone, dimethylmethcathinone DMMC, flephedrone. Substances from other classes were diphenidine, encountered for the first time in Europe, NM2AI (N-methyl-2aminoindane), MPA (1-(thiophen-2-yl)-2-methylaminopropane), MPPA (4-methyl-alpha-pirrolidinopropiophenone), MTTA (mephtetramine), Bk-2CB (2-amino-1-(4-bromo-2,5-dimethoxyphenyl)ethan-1-one). Only three seizures contained synthetic cannabinoids, and were herbal material soaked with N-(1-adamantyl)-1-pentyl-1H-indazole-3-carboxamide (AKB 48), or with a mixture of 5-fluoro-AKB and BB-22 (1-(cyclohexylmethyl)-8-quinolinyl ester-1H-indole-3-carboxylic acid). In some cases there were mixtures of drugs, such as granules containing 4-MEC and pentedrone, in one case with traces of diphenidine, in other cases 5-MeO-DALT, ethylphenidate and caffeine, in one case flephedrone and methoxethamine, in one case methylone, ethylone, methedrone, 4-Fluoroamphetamine, 5-MethoxyDALT and 5MeO-MIPT. Six shipments from Philippines contained crystals of pure methamphetamine. 9 seizures were tablets containing sildenafil.
The proposed analytical approach allowed the identification of NPS in seizures, also in absence of an analytical standard. The analyses performed showed the presence in the Italian territory of a wide number of NPS coming from other EU Countries. This confirms the threat for public health, especially when NPS are sold in combination and when wrong or missing information are reported on the label.

P15. SIMULTANEOUS DETERMINATION OF 36 NOVEL DESIGNER DRUGS OF AMPHETAMINES AND THEIR ANALOGUES IN URINE AND HAIR BY LC-MS-MS

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The extraction and detection methods of new designer drugs of amphetamines and their analogues (they are classified as phenylisopropylamines compounds) from urine or hair samples are in great need due to these new drugs are abused severely in recent years all over the world. Highly sensitive analytical techniques are therefore required for trace-level identification and quantification of these kinds of drugs.
The aim of the research was the validation of simultaneous identification of 36 phenylisopropylamines in urine and 13 phenylisopropylamines in hair by liquid chromatography tandem mass spectrometry (LC/MS/MS).
Phenylisopropylamines from spiked urine or hair specimens were analyzed by LC/MS/MS with different pretreatments as follows: 1.Urine Specimens: a 0.2 ml portion of urine was adjusted to pH 9.0 with 0.1N phosphate buffer. For solid-phase extraction, the urine sample was then diluted with 2.5 ml of water and loaded on a Ware Trace-B cartridge, previously conditioned with 1 ml of methanol and 1 ml of water. After passage of the sample, the cartridge was washed with ethyl acetate/methanol/ammonia mixture (80:20:2 v/v). The eluate was dried in N_2 at 45°C, and the residue was dissolved in 0.2 ml of mixture of water/methanol/ formic acid (90/10/0.1 v/v). Gradient elution was performed by an Agilent Zorbax SB-Aq (3.5 um, 2.1x150 mm) analytical column for LC/MS/MS instrumental analysis. 2. Hair Specimens: a 20 mg portion of hair sample was added with 0.2 ng/mg deuterated internal standard and incubated at 45°C for 18 hours with 0.1N HCl for dissolving the hair sample. For liquid-liquid extraction, the dissolved hair sample was extracted with 0.1mL 1N NaOH and 0.4 mL ethyl acetate. The organic solvent layer was transferred and gently evaporated to dryness in N_2 at 40°C. The residue was dissolved in 0.2 ml mixture of 0.1% aqueous formic acid and 0.1% formic acid in methanol. Gradient elution was performed by an Agilent Zorbax SB-Aq (3.5 um, 2.1x150 mm) analytical column for LC/MS/MS instrumental analysis.
Validation of qualitative and quantitative analysis of phenylisopropylamines from spiked urine and hair samples by LC/MS/MS was performed. Over 135 authentic urine and hair specimens were analyzed. 1.Urine Specimens: 36 Phenylisopropylamines from spiked urine specimens were analyzed by LC/MS/MS. The limits of detection (LOD) range from 0.05 ng/mL to 1 ng/mL and the limits of quantification (LOQ) range from 0.25 ng/mL to 5 ng/mL. Linearity was in the range from 5 ng/mL to 100 ng/mL, for each compound (R^2 from 0.9945 to 0.9997). Mean relative errors were between ±10.0%. Precision variance was less than 15%. 2. Hair Specimens: 13 Phenylisopropylamines from spiked hair specimens were analyzed by LC/MS/MS. The limits of detection (LOD) range from 0.01 ng/mg to 0.05 ng/mg and the limits of quantification (LOQ) range from 0.02 ng/mg to 0.1 ng/mg. Linearity was in the range from 0.1 ng/mg to 2.0 ng/mg, for each compound (R^2 from 0.9950 to 0.9998).Mean relative errors were between ±10.0%. Precision variance was less than 15%.
Highly specific qualitative and quantitative analysis of 36 novel designer drugs of amphetamines and their analogues in urine and hair by LC/MS/MS has been validated and successfully applied to real samples.

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P16. COMPARISON OF HPLC-QTOF MS/MS ANALYSIS TO A ROUTINE EMIT, HPLC, GC/NPD AND GC/MS WORKFLOW FOR FORENSIC DRUGS OF ABUSE SCREENING

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Recent advances in QTOF hardware and software have increased the feasibility of applying this technology to routine forensic drugs screening. The Wisconsin State Laboratory of Hygiene (WSLH) Toxicology Section currently uses multiple methods (EMIT, HPLC (with wavelength detection) and GC based screening) to analyze for more than 300 drugs of abuse compounds in over 18,000 medical examiner and operating while intoxicated samples each year. With recent illicit drug use trending towards newer drugs, such as synthetic cannabinoids and other novel psychoactive substances, the number of possible analytes makes their identification and confirmation difficult.
The goal of the present study was to investigate the use of HPLC coupled to QTOF detection for both targeted and unknown identification of drugs of abuse screening workflows and to compare results observed with the suite of current screening methods used in the WSLH Toxicology Section.
Ten blinded whole blood samples previously analyzed by the EMIT, HPLC, GC/NPD and GC/MS workflows at the WSLH were extracted via protein precipitation and shipped to SCIEX (Redwood City, California) and analyzed by a Shimadzu Prominence HPLC coupled to a SCIEX TripleTOF® 5600+ LC-MS/MS system with both TOF-IDA-MS/MS and TOF-MS/MS-All data acquisition with SWATH™ acquisition modes in non-targeted fashion.
A total of 112 compounds were detected using both methods. Seven compounds reported by the EMIT, HPLC, GC/NPD and GC/MS workflows were not reported in the blinded HPLC-QTOF MS/MS analysis. This is likely due to the difference in sample preparation procedures used. The WSLH GC base screening extraction procedure uses a double solvent extraction/cleanup and the samples for the HPLC-QTOF MS/MS analysis were prepared by a simple protein precipitation procedure and were further diluted 1:10 prior to analysis. Moreover, the HPLC-QTOF MS/MS analysis also detected five compounds with high confidence using MS/MS matching that were not detected with the original WSLH screening analysis methods.
The use of HPLC-QTOF MS/MS shows great promise for streamlining routine drugs of abuse screening workflows and has the added benefit of being able to detect and identify true unknowns thus making this a very powerful analysis technique for forensic toxicology laboratories.

P17. DEVELOPMENT AND VALIDATION FOR A HIGHLY SENSITIVE GC-MS/MS SCREENING AND QUANTIFICATION METHOD OF FORENSICALLY RELEVANT DRUGS OF ABUSE IN WHOLE BLOOD

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With fatal poisoning cases, forensic toxicologists are faced with the challenges of identifying and quantifying drug blood concentration. Trends in forensic toxicology show the advancement of rapid and sensitive analytical methods for qualitative and quantitative analysis of drugs of abuse. We have developed an identification and quantification method that is simple, robust, and highly sensitive by GC-MS/MS equipped with MRM and quantification database. The method was applied to real case samples and was proved to be effective.
The development and validation of a GC-EI-MS/MS method with high sensitivity and quantitative performance of forensically relevant drugs of abuse in blood, operated in the MRM mode is discussed.
Whole blood samples (0.5 mL) were spiked with standard compounds of 30 commonly analyzed benzodiazepines (20), tricyclic and tetracyclic antidepressants (10) at 1-750 ng/mL concentrations. Deuterium-labelled analogues of a representative drug from each drug group were used as internal standards (IS). Quintuplicate samples were prepared by QuEChERS extraction. Samples were reconstituted with 50 µL of ethyl acetate and analyzed by GCMS-TQ8040 (Shimadzu) in the MRM mode. Optimal CE for each MRM transition was determined using the MRM Optimization Tool (Shimadzu) for all compounds as follows: after first obtaining single EI scan mass spectra, three ions were selected as precursor ions. Product ion spectra were obtained for these ions, followed by MRM transition optimization for each by varying the collision energy (CE) from 3 to 45 V in 3 V intervals to determine the optimal CE for each MRM transition (one for quantitation and two for confirmation). This procedure was repeated for the deuterated analogues to be considered for IS. Deuterated compounds that only produced product ions identical to their non-deuterated counterparts were omitted from the study. I dentification and quantification of the drugs were conducted with a GCMS Solution software and modified Smart Forensic Database (Shimadzu). Validation parameters were determined according to the international guidelines.
QuEChERS extraction provided adequate recovery rates of the target drugs ranging between 60-98%, except for etizolam (45%), chlorpromazine (28%), and promethazine (40%). Although manual determination of optimized MRM transitions is, especially in EI-MS/MS condition, MRM Optimization Tool was effective and allows objective optimization. Spiked blood samples were analyzed with the developed method, and of the 30 investigated drugs, 15 were detectable at 1 ng/mL and 10 were detectable at 5-10 ng/mL, demonstrating the highly sensitive identification ability of the method. Four benzodiazepines and two tetracyclic antidepressants could not reach sufficient sensitivity in their free form thus are under consideration for derivatization. Calibration curves were linear over the specified range with R^2 values >0.99. Sufficient validation results were obtained for majority of the investigated compounds.
A GC/MS/MS quantitation method in combination with QuEChERS extraction was developed. This method can detect ng/ml-order levels of target drugs in blood with high specificity. Since its sensitivity and quantitativity is equal to a middle-range LC/MS/MS system, this method can also be used for cross-check of the results in real forensic cases. The robustness and usability of the system based on GC/MS/MS is also advantageous for forensic toxicologists.

P18. **SENSITIVE LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY METHOD FOR THE SIMULTANEOUS DETERMINATION OF 9 KINDS OF LOCAL ANESTHETIC DRUGS**

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Intoxication by the anesthetic drug compared with the other drugs significantly lower but often occurring by its medical accident or malpractice, has become a major social problem. Local anesthetic drug can be classified into ester-type and amide-type drugs according to their structures. Both types of the drugs differ in the mode of metabolism and chemical stability. Local anesthetic drug by applicable law surface anesthesia, anesthesia, transfer anesthesia, spinal anesthesia, epidural also should note that can increase blood concentrations of local anesthetic drug toxicity is divided into anesthesia for many medical accidents in the present situation.
The aim of this study is to develop and validate a sensitive liquid chromatography-tandem mass spectrometry (LC/MS/MS) with ESI method for the simultaneous determination of 9 kinds of local anesthetic drugs (bupivacaine, dibucaine, lidocaine, mepivacaine, oxybuprocaine, procaine, ropivacaine, T-caine, tetracaine).
An Oasis® HLB cartridge column (1 mL, Waters) was placed in a Vac-Elut® system. The human serum was added cholinesterase inhibitor, such as neostigmine bromide. The 1 mL of human serum containing 25-100 ng of each of 9 kinds of local anesthetic drugs and 100 ng of lidocaine-d10 (IS) were added to 5 µL phosphoric acid and 500 µL water. The column was then washed with 1 mL of 10% methanol - 2% ammonium hydroxide in water solution. The local anesthetic drugs were eluted with 1 mL of 2% acetic acid in methanol solution. The eluent was dried under N_2 gas at 40˚C. The residue was dissolved in 200 µL mobile phase and aliquot (10 µL) was injected into the LC/MS/MS. LC/MS/MS was performed on a API-4000™ system (AB SCIEX). The LC column was a Mightysil-RP-18 GP µ column (2.0 mm x 150 mm, particle size 5 µm). For gradient elution, two solvents were used : (A) acetonitrile and (B) 10 mM acetic ammonium buffer. The 65% B was held for 10 min. Linear gradient elution was started from 65% B to 40% B over 20 min. It was then returned to 65% B over 5 min for the next run. The flow rate was 0.20 mL/min and the column temperature was maintained at 37˚C. The settings of the turbo ion-spray were as follows; curtain gas, N_2:40 psi, collision gas, N_2:4 psi, collision energy:27~41V, ion-spray voltage:5500 V, source temperature:600˚C. Separation and sensitivity for the detection of 9 kinds of local anesthetic drugs by LC/MS/MS were sufficient. In addition, $[M^+H]^+$ ion connection peak was detected in the mass spectrum of each drug clearly. The calibration curves were linear over the concentration range of 25-100 ng/mL for these drugs. The extraction yields of 1 mL human serum sample with Oasis® HLB cartridge column were good and the limit of quantification were 0.04-0.42 ng/mL respectively.
The presently established method is very useful for simultaneous measurements of local anesthetic drugs in human serum by LC/MS/MS.

P19. **EVALUATION OF THE WATER FLUORIDE EXPOSURE IN URBAN AREAS IN THE PROVINCE OF BLIDA, ALGERIA**

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Fluoride (F-) is an oligo-element which prevents tooth decay, but it is toxic in high doses. In Algeria, tooth decay is the most common disease in the school environment; on the other hand, the fluorosis problem affects many areas of southern and even northern Algeria because of the ingestion of excess fluoride, most commonly in drinking-water.
Our study focuses on the evaluation of the water fluoride exposure in the population of Blida city, based on the determination of fluoride in the municipal water distribution network, in order to predict the possible risk of fluorosis, or orient the public health programs related to fluoride supplementation.
30 water samples were collected during the period from 31 March to 04 April 2014 in three regions of Blida. We realized the determination of fluoride by a colorimetric method (Belcher West) that we have validated according to the protocol of the French Society of Technical Sciences and Pharmaceutical SFSTP'92.
All the validation's parameters required by the protocol SFSTP'92 are valid which demonstrates the performance and reliability of the method of Belcher West used for the determination of fluoride in water. The concentrations of F- in water through Blida are below the Algerian drinking-water standard (0.5-2 mg /L) and the World Health Organization (WHO) drinking-water standard (0.8-1.7 mg/L), the lowest values are observed in the eastern region (L'Arba) with 60% of the values <0.05 mg / L, followed by the central region (Blida) with 70% of the values <0.17 mg /L and finally the Western region (Elafroune) with 60% of the values <0.17 mg / L.
Based on these results we can conclude that there is no risk of fluorosis in Blida city on the contrary the values found are very low, hence the need to establish a fluoride supplementaion program in school children by administration of tablets containing sodium fluoride in order to prevent tooth decay.

Poster abstracts

P20. **QUANTIFICATION OF COCAINE, COCAETHYLENE AND ANHYDROECGONINE METHYL ESTER (AEME) IN WHOLE BLOOD USING HOLLOW-FIBRE LIQUID PHASE MICROEXTRACTION (HF-LPME) AND GAS-CHROMATOGRAPHY MASS-SPECTROMETRY (GC-MS)**

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According to the statistic data provided by the United Nations Office on Drugs and Crime, Brazil today consumes 18% of the world's yearly supply of the cocaine. Estimates have shown that Brazil's coke explosion has been called "the most worrying side-effect of the country's recent consumer boom," with its use spreading not only across slums, known as "cracolândias", but also among the swelling middle class, who now comprises of more than half of the population. Consequently, this contributes to the high number of deaths by overdose of this drug and accidents.
A method for the determination of cocaine, cocaethylene and anhydroecgonine methyl esther (AEME-crack biomarker) in whole blood samples was developed in the present work, using hollow fiber liquid phase microextraction (HF-LPME) and gas chromatography-mass spectrometry (GC-MS).
An aliquot of 200 µL whole blood was pippeted into a 1.5 mL eppendorf tube, followed by the addition of 15 µL of potassium hydroxide (0.05 M) along with sodium chloride (200 mg) and filled up to approximately 1.5 mL with deionized water. A 9-cm of hollow fiber was immersed into diexyl ether, filling its pores with the solvent. The fiber was then filled with acceptor phase consisting of hydrochloric acid (5 mM) and introduced into the sample solution. The extraction was performed by lateral vortex mixing the solution for 15 min at 2400 rpm. After extraction, the acceptor phase was withdrawn from the fiber and dried under nitrogen stream (40°C). The residue was re-suspended in 50 µL of ethyl acetate and injected into the GC-MS. Cocaine-d3 was used as internal standard and UNODC guidelines were followed.
The limit of quantification (LoQ) was 10 ng/mL for all analytes. The calibration curves were linear over a concentration range of 10.0 ng/ml to 1200 ng/ml (R^2 = 0,99). The method showed to be precise (RSD <15%) and sensitive with a recovery value of 56%. A total of three real samples have been tested from which two of them yielded a positive result for cocaine.
This method has allowed not only for the determination of low amounts of cocaine, cocaethilene and AEME at once but also for the application of an environmental and cost-friendly HF-LPME technique. When compared to conventional extraction techniques such as LLE or SPE, this microextraction has proven to be much faster, significantly less harmful due to its reduced use of hazardous solvents, sensitive considering the low volume of bloof required for analysis and of general easy clean-up.

P21. **DEVELOPMENT OF THE FIRST POLYCLONAL ANTIBODY FOR THE DETECTION OF TILIDINE AND ITS ACTIVE METABOLITE NORTILIDINE**

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Tilidine [(1R, 2S)-2(dimethylamino)-1-phenyl-3-cyclohexane-1-carboxylic acid ethyl ester] or tilidate is a synthetic opioid analgesic, used mainly is Germany, Switzerland, South Africa and Belgium for treatment of moderate to severe pain, both acute and chronic. It is also used in combination with the opiate antagonist naloxone to prevent drug abuse. Tilidine is a prodrug from which the active metabolite nortilidine is formed by demethylation and the secondary metabolite is bisnortiline. The most common adverse effects are nausea and vomiting, dizziness, drowsiness, fatigue, headache and nervousness, but it has also been reported to cause fatalities. Tilidine is a Schedule I narcotic controlled substance in the United States, as defined by the United States Controlled Substances Act.
The aim of this study was to develop the first polyclonal antibody to the synthetic opioid, tilidine and its active metabolite nortilidine. This antibody could be used in the development of immunoassays for the detection and quantification of tilidine and nortilidine in biological samples (urine, blood, oral fluid and hair samples).
The tilidine hapten was synthetized in multi-steps and conjugated to a carrier protein Bovine Thyroglobulin (BTG) for the preparation of the immunogen, using standard methods of conjugation. The immunogen was administered to adult sheep on a monthly basis to provide target-specific polyclonal antiserum. IgG was extracted from the antiserum and evaluated via competitive Enzyme-Linked Immunosorbent Assay (ELISA).
In this initial evaluation the assay was standardised against tilidine. The active metabolite nortilidine was also detected with a cross-reactivity value of 21.35%. Assay sensitivity, expressed as a half maximal inhibitory concentration (IC_{50}) was 1.82ng/mL (assay range 0-40.0ng/mL).
The data indicates that the developed polyclonal antibody detects the synthetic opioid analgesic tilidine and its active metabolite nortilidine. This antibody is suitable for the development of immunoassays for the detection and quantification of tilidine and nortilidine in human biological samples for forensic and toxicology applications.

P22. EVALUATION OF A NEW ELISA KIT FOR THE SCREENING OF THE EMERGING SYNTHETIC CANNABINOID AB-PINACA IN BLOOD AND URINE

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AB-PINACA [(S)-N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-pentyl-1H-indazole-3-carboxamide] was first identified as a synthetic cannabinoid present in illegal herbal products in Japan in 2012.The Drug Enforcement Administration recently issued a final order to temporarily schedule AB-PINACA into schedule 1 of the Controlled Substances Act to avoid an imminent hazard in the public safety. It is important to have new screening technology available to coincide with the emergence of new synthetic cannabinoids.
The aim of this study was to evaluate a new Enzyme-Linked Immunosorbent Assay (ELISA) for the detection of AB-PINACA in whole blood and urine. In addition, urine samples from the National Institute of Occupational Safety and Health (NIOSH) were assessed.
A competitive colorimetric immunoassay was employed. The capture antibodies were immobilized and stabilized on a 96-well microtitre plate surface. The analyte, if present in the sample, competes with the horseradish peroxidase labelled conjugate for antibody binding sites on the microtitre plate. Assay specificity was calculated by determining the IC50 of the standardising antigen compared to that of the cross-reacting compound. The limit of detection (LOD) was calculated from the mean concentration of 20 negative samples (blood and urine) +3SD. Accuracy was determined by spiking AB-PINACA N pentanoic acid into negative urine and blood samples at 3 concentration levels (5ng/mL cut-off and +/-50% of this cut-off). Three replicates of these samples were assessed across 5 different runs and the mean concentration, % recovery and inter-assay precision were calculated. Additionally urine samples from NIOSH were assessed, they included: 5 confirmed negative samples, 20 field samples with unknown AB-PINACA present and 56 samples containing increasing spiked levels of AB-PINACA, AB-PINACA pentanoic acid, AB-PINACA 4OH pentyl and AB-PINACA 5OH pentyl.
The ELISA was standardised to AB-PINACA N pentanoic acid and the assay also detected 13 AB-PINACA compounds with cross-reactivity values >5% (from 5 fluoro ADB-PINACA at 9.8% to 5 fluoro AB-PINACA at 98.9%). LOD in blood was 0.41ng/ml (assay range:0-20ng/mL) and 0.26ng/mL in urine (assay range:0-12ng/mL). The recovery(%) was calculated to be 87% at 50% below the cut-off, 91.2% at the cut-off of 5ng/ml and 117.47% at 50% above the cut-off. The inter-assay precision expressed as CV(%) (n=15) was<10% at all levels. The assessment of the urine samples from NIOSH with this ELISA kit showed: the five confirmed negative samples were screened as negative (below cut-off 5 /mL), from the 20 field samples 5 were screened as positive (above cut-off 5ng/mL), the assessment of 56 spiked samples showed a 100% agreement when compared with the spiked concentrations.
This evaluation indicates applicability of this new ELISA kit to the detection of the synthetic cannabinoid AB-PINACA and related compounds in blood and urine. The LODs for both matrices were below the cut-off concentration of 5ng/mL. The assessment of urine samples from NIOSH with this ELISA showed optimal concordance. This ELISA represents a useful screening tool for forensic and toxicology applications.

P23. NEW ACQUISITION AND PROCESSING TOOLS FOR TARGETED AND UNKNOWN SCREENING APPROACHES IN TOXICOLOGY AND FORENSIC

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Toxicology and forensic laboratories require sensitive and selective analytical methods to properly identify a broad range of analytes in complex matrixes. Existing methods need to be constantly updated as new compounds are available every day on the market. Liquid chromatography coupled to mass spectrometry has been widely used in this area for years, the preferred technology being triple quadrupoles for their selectivity in SRM (Selected Reaction Monitoring) mode. This approach, however, can only perform targeted analysis and not unknown screening; the use of High Resolution and Accurate Mass (HRAM) instruments has gained in popularity by offering the opportunity to perform screening in full scan mode and identify new substances. The two main technologies used for screening purposes are the Time of Flight and the Orbitrap™, the latter providing superior resolution and very good mass accuracy without need for an internal mass calibrator.
To introduce two new analytical approaches based on Orbitrap technology to support targeted and unknown screening.
The first analytical approach is data-dependent acquisition (DDA): the most intense ions identified in full scan mode are isolated, fragmented and fragments detected in the Orbitrap; this is the most popular analytical approach for targeted screening. A new HRAM scan mode, termed variable data-independent acquisition (vDIA) can be applied to both targeted and unknown screening: MS/MS fragmentation is performed on a number of m/z isolation windows covering the entire mass range of the preceding full scan; this approach is required to identify drugs that are not available in databases and spectral libraries such as new designer drugs.
Data acquired using DDA are processed using a database in combination with a spectral library, provided with TraceFinder™ software, containing up to now more than 1500 analytes; identification and/or confirmation of an analyte is based on its exact mass, the isotopic pattern, the fragment ions, the retention time and the mass spectrum. This analytical approach allows for improved sensitivity and specificity. Data acquired using a vDIA approach are processed using mzCloud™ and Mass Frontier™. mzCloud is a modern MSn spectral database searchable from spectra, structures, fragment and precursor ions allowing compound identification even if they are not present in the database. Mass Frontier is available for structural elucidation: it is able to automatically generate possible

fragments at an expert level, including complete fragmentation and rearrangement mechanisms, starting from a user-supplied chemical structure. The knowledge base used to predict fragmentation reactions consists of 24 ionization, fragmentation and rearrangement rules along with more than 100,000 published fragmentation mechanisms.
New tools such as vDIA, mzCloud and Mass Frontier combined with TraceFinder software provide both targeted and unknown screening capabilities for identification of a broad range of analytes in complex matrixes.

P24. SCREENING OF FASCICULOL E AND F AND ILLUDIN S IN MUSHROOMS

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In Japan, mushroom poisoning often occurs because of incorrect picking during spring and autumn. Hypholoma sp. And Omphalotus japonicas are known to be highly toxic, and several cases of accidental death because of the consumption of these mushrooms have been reported in Japan, most often because of O.japonicas. The toxic components of Hypholoma and O. japonicas are fasciculole E and F and illudin S, respectively; the detection of these compounds in body fluids, leftover food, or vomit plays an important role in the clinical and forensic toxicology investigation in case of suspected mushroom poisoning by natural poisoning. However, these compounds are not easily detectible in blood or urine.
We have developed a method for rapid detection of fasciculol E and F and illudin S in mushrooms using liquid chromatography-tandem mass spectrometry (LC-MSMS).
Mushroom (1g) were mixed with water and homogenized or vortexed and then centrifuged for 3 min at 3,000 rpm. The obtained supernatant was applied to the InertSap® diatomite column. Analytes were eluted with methanol and injected into the LC-MS/MS system without drying. For separation, a 3-µm × 100 mm InertSustain C18 HP column was used. Gradient elution, using 0.1% acetic acid aqueous solution and acetonitrile, was performed for chromatographic separation. For MS/MS analysis, two ion transitions were monitored for each analyte: fasciculol E and F m/z 724.5→234.1 and m/z 724.5→437.3 and illudin S m/z 265.2→217.1 and m/z 265.2→95.1.
The comparison of the chromatograms of the control mushroom (Lentinula edodes), Hypholoma, and O. jeponicus did not show interfering peaks eluting at the same retention times for any of the three analytes, confirming that the applied chromatographic conditions were satisfactorily selective. Although the three compounds were appropriately detected, fasciculol E and F could not be separated on the InertSustain® column because they are diastereomers. Thus, this method could be successfully applied to a clinical mushroom poisoning case.
In the present method, the sample preparation procedure and LC-MS/MS analysis takes approximately 20 min. To our knowledge, this is the first report describing rapid the identification of fasciculol E and F and illudin S from mushrooms.

P25. STRATEGIES TO UNCOVER THE ADMINISTRATION OF RECOMBINANT HUMAN INSULIN BY LC-HRMS

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The use of insulin for performance enhancement in sport or as toxic agent has frequently been reported in the past. In contrast to the different synthetic insulin analogues, the administration of recombinant human insulin is hardly recognized by mass spectrometry.
The present study was designed to uncover the misuse of recombinant human insulin for doping control purposes as well as for forensic applications. The hypothesis is a presumably altered metabolite profile of circulating insulin prevails after subcutaneous administration due to exposure of insulin to proteases in the epidermal tissue.
In-vitro experiments with skin tissue lysates (S9 fraction and microsomes), different biological fluids (urine, serum, plasma) and recombinant human insulin were performed and the deriving metabolites were characterized by liquid chromatography coupled to high resolution mass spectrometry (HRMS). Afterwards, real blood samples of patients suffering from diabetes mellitus and a control group of healthy humans were analyzed. Therefore, a method using protein precipitation, ultrafiltration and antibody-coated magnetic beads for purification with subsequent separation by nano-scale liquid chromatography coupled a QExactive-HRMS was applied.
Several metabolites of insulin with C-terminal truncated sequences of the B-chain (and A-chain in minor extent) were identified within this study. Additionally, the cleaved B-chain and its truncated form was detected in-vitro. Here, the DesB30 human insulin represents the major metabolite in all experiments. This metabolite is frequently found in urine samples due to degradation processes and, thus, disqualifies this matrix for diagnostic analysis. Blood samples normally do not contain DesB30 insulin which was additionally shown in the control group. The highly sensitive analysis of the post administration blood samples yielded trace amounts (< 50 pg/mL) of DesB30 insulin and indicates this target peptide as marker for human insulin administration.
The detection of insulin metabolites in blood samples represents a potential marker to uncover an illicit rec. human insulin administration.

P26. ESTIMATION OF THE STRUCTURE OF SYNTHETIC CANNABINOIDS USING GC–EI–MS AND GC–PCI–MS

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Various illegal drug products having cannabimimetic properties have recently become commercially available via the Internet and street shops in Japan. Many drug compounds in Japan known as "dangerous drugs" have been identified in herbal products and are regulated as scheduled substances (Shitei–Yakubutsu) by the Pharmaceuticals Affairs Law of Japan. However, new compounds successively continue to appear without administrative regulations. In 2014, these compounds were responsible for 112 deaths in Japan. Further, when a new compound is discovered, its structural determination can be difficult.
In the present study, we report the use of GC–MS–EI and GC–MS–PCI for investigating the structures of two types of synthetic cannabinoid: naphthoylindole and nephethoylindasol.
MAM-2201, NM2201, NNEI, and XLR-11 N-(4-fluoropentyl) isomers were used as naphthoylindole compounds. FUD-AMB, MDMB-CHMINACA, MDMB-FUBINACA, 5-fluoro AB-PINACA, and AB-CHMINACA were used as standard naphethoylindasol compounds. All standard reagents were analyzed using GC–MS–EI and GC–MS–PCI in the scan mode. Moreover, methane was used as the reagent gas for PCI. GC–MS was performed using an Agilent 6890 N GC system with a 5975B mass selective detector and an HP-5MS column (30 m × 0.25 mm i.d., 0.25-µm film thickness).
EI: The carbonyl group fragment ions, which arise from the α-cleavage of the alkylamino group of the indole and indasol in naphthoylindoles and naphethoylindasols, respectively were observed as base peak. Fragment ions at m/z 144 or 145 were observed in naphthoylindoles and naphethoylindasols, respectively, similar to those observed in N-dealkylation. PCI: M+1 was found as the base peak, and M-16 (-NH_2), M-31 (-O-CH_3), M-44 (-CO-NH_2), and M-59 (-CO-O-CH_3) were observed as fragments. Retention times were the same for EI and PCI. Although NNEI was detected only with EI, all other compounds were detected using both ionization modes. These results were successfully applied to unknown compounds in herbal products.
GC–MS–EI and GC–MS–PCI procedures were evaluated for estimating the structure of synthetic cannabinoids. These methods can be efficiently applied for elucidating the structure of unknown synthetic cannabinoids.

P27. SIMULTANEOUS DETERMINATION OF MUSHROOM TOXINS α-AMANITIN, ß-AMANITIN AND MUSCARINE IN HUMAN URINE BY SOLID-PHASE EXTRACTION AND ULTRA-HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED WITH ULTRA-HIGH-RESOLUTION TOF MASS SPECTROMETRY

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Muscarine is the principal toxin in fungi of the genus Inocybe, Clitocybe and together with isoxazole derivatives ibotenic acid and muscimol is also present in the genus Amanita (Amanita pantherina, Amanita muscaria and others). The Amanita phalloides poisoning is rare, but may cause severe or even fatal intoxication. Amanita mushrooms contain amatoxins such as α-amanitin and β-amanitin and phallotoxins such as phallacidin. Muscarine was used as a diagnostic marker for poisonings of Amanita pantherina and Amanita muscaria. α-Amanitin and β-amanitin were used as diagnostic markers for Amanita phalloides poisoning. Phallacidin was chosen as an internal standard (IS) because it is not absorbed from the intestine.
The aim of the present study was to develop a fast and sensitive method for simultaneous analysis of α-amanitin, β-amanitin and muscarine in human urine by solid-phase extraction (SPE) and ultra-high-performance liquid chromatography coupled with ultra-high-resolution TOF mass spectrometry.
The urine sample was centrifuged at 13400 RPM for 5 min and the supernatant was collected. For the sample preparation by SPE on Strata X-CW 30 mg cartridge was used. All analyses were carried out using a UHPLC UltiMate 3000 RSLC System (Dionex) connected with a UHR-TOF Maxis Impact HD (Bruker Daltonics). Chromatographic separations were performed at 30 °C on a reverse phase analytical column Acclaim RS 120, C18 2.2 µm, 2.1 x 100 mm (Thermo Fisher Scientific, Waltham, MA, USA). The chromatographic conditions used were: injection volume 5 µl; flow rate 0.2 mL/min; mobile-phase solvents (A) H_2O/acetonitrile 99/1 with 2 mM ammonium formate and 0.1 % formic acid and (B) acetonitrile/H_2O 99/1 with 2 mM ammonium formate and 0.1 % formic acid. Gradient of (B) from 1 to 90 % was used.
Partial validation was performed on spiked human urine samples. Limits of detection, linearity, intraday and interday precisions and recoveries were determined. The obtained LOD values of α-amanitin and β-amanitin were 1 ng/mL and of muscarine 0.09 ng/mL. All calibration curves exhibited good linearity in the range 0.1-100 ng/mL for muscarine and 1-1000 ng/mL for α-amanitin and β-amanitin. The intraday and interday precisions of human urine spiked with α-amanitin (10 ng/mL), β-amanitin (10 ng/mL) and muscarine (1 ng/mL) ranged from 6 % to 10 % and from 7 % to 13 %, respectively. Finally the urine samples from 28 patients suspected for mushroom poisoning were analyzed. Mushroom intoxication was confirmed in two cases. In both cases the results were confirmed by simultaneous analyses of the blank urine and the urine spiked with muscarine (1 ng/mL) and α-amanitin (10 ng/mL), respectively.
A UHPLC-UHR-TOF MS method for the simultaneous determination of α-amanitin, β-amanitin and muscarine in human urine was developed, partially validated and successfully applied to samples of intoxicated patients.

Poster abstracts

P28. **DERIVATIZATION OF 15 CATHINONE ANALOGS WITH 2,2,2-TRICHLOROETHYL CHLOROFORMATE - A GAS CHROMATOGRAPHY - MASS SPECTROMETRY STUDY**

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The market growth of new psychoactive substances is a complex challenge that affects both health and safety of citizens worldwide. In particular, an increasing number of new amphetamine-related designer drugs, including cathinone analogs, are appearing on the recreational drug market. Obtaining the structural characterization of these new drugs and detect them in biological and non biological samples has been one of the recent goals of clinical and forensic toxicology laboratories.
Pursuing our interest in the analysis of amphetamine-related drugs after derivatization with 2,2,2-trichloroethyl chloroformate and with the aim of improving the potential of identification of cathinone analogues, we employed the above derivatization agent for 15 cathinone analogs, namely methcathinone, mephedrone (4-MMC), 3-methyl-methcathinone (3-MMC), methedrone, flephedrone, pentedrone, 3,4-dimethylmethcathinone, ethcathinone, buphedrone, 4-methylethcathinone (4-MEC), methylone, ethylone, butylone, pentylone, bk-2C-B.
Cathinone analogs were analyzed by gas chromatography – electron impact/mass spectrometry (GC-EI/MS) after derivatization with a mixture of 2,2,2-trichloroethylchloroformate:ethyl acetate (3:7) at 80°C for 15 min. GC- EI/MS analyses were performed on a Agilent 7890 series II/5975 GCMS system in EI full-scan (m/z 40-600) conditions with an Agilent HP-5MS UI (30 m x 0.25 mm, 0.25 µm film thickness) capillary column. Oven temperature was programmed from 50°C (0.5 min) to 200°C at 30°C/min, then to 300°C (5 min) at 10°C/min.
Due to the low molecular weight and relatively high polarity of underivatized cathinone analogs, their GC peaks are characterized by fairly short retention times and possibly widening and tailing, thus hindering GC resolution. Furthermore, their EI mass spectra are quite non-specific due to the barely visible molecular ions and the base peaks at m/z 44, 58, 72, 86, and 100, caused by the well-known formation of the iminium ions. The straightforward and relatively rapid derivatization of the 15 cathinones with 2,2,2-trichloroethylchloroformate allowed to obtain main features of a twofold nature: (a) great enhancement of the molecular weight of the original analytes (+174 Da), leading to new compounds with changed polarity and volatility properties; this allows improved overall MS selectivity and GC properties, such as relatively long retention times and non-tailing peak shapes, resulting in potentially better chromatographic selectivity when analyzing complex biological matrices; (b) introduction of three chlorine atoms in the derivatized molecules, giving rise to distinctive MS signatures; the presence in many cases of detectable molecular ions, and in all cases of abundant fragment ions with characteristic isotopic clusters, permits unambiguous analyte identification. These features easily allow also the analytical discrimination of cathinones with the same molecular formula, e.g. buphedrone, 3-MMC, 4-MMC (all $C_{11}H_{15}NO$).
The derivatization with 2,2,2-trichloroethylchloroformate can improve the potential of identification and quantitation of cathinone analogs in both biological and non biological samples, and may result particularly useful to laboratories that do not have access to MS techniques different from GC-EI/MS.

P29. **ETHANOL WORKBOOK (ETWB): A USER-FRIENDLY SOFTWARE APPLICATION FOR MEASUREMENT UNCERTAINTY CALCULATION, DATA MANAGEMENT, COMPLIANCE ASSESSMENT AND QUALITY CONTROL OF FORENSIC BLOOD ALCOHOL CONTENT DETERMINATIONS**

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The measurement of blood–alcohol content (BAC) is a crucial analytical determination required to assess if an offence (e.g. driving under the influence of alcohol) has been committed. Reliability of results is then a critical issue and measurement uncertainty, significant figures and rounding errors become relevant topics for assessing compliance to specification limits as they directly affect the final result. To handle this a certain amount of data processing is usually required which may be complex and time consuming.
A software application named Ethanol WorkBook (EtWB) was developed at the authors' laboratory with the aim of providing help to forensic analysts involved in BAC determinations. The main goal of the tool is to facilitate calculations which could be error-generating and time-consuming in the routine laboratory procedures.
EtWB has been developed by using Visual Basic for Application language and MS Excel®. The program makes large use of graphical interfaces (userforms), thus providing user–friendly accessibility.
The program can (i) calculate measurement uncertainties and decision limits with different methodologies; (ii) assess compliance to specification limits with a guard band approach; (iii) manage quality control (QC) data and create control charts for QC samples; (iv) create control maps from real cases data archives; (v) provide laboratory reports with graphical outputs for elaborated data; (vi) create comprehensive searchable case archives. The basic workflow of the program is as follows: 1) the user is asked to login and assign a sample ID; 2) the user enters the BAC measurements obtained for that ID; 3) calculations are performed by the program providing statistics, charts and results; 4) the user is asked to validate obtained results to proceed and archive data; 5) a new cycle can then be initiated by entering a new ID. Several options have been also included to provide a range of custom settings.
EtWB has been specifically designed to help analysts involved in blood alcohol content determinations for forensic purposes. EtWB covers the needs of the authors' laboratory but can be useful for many other forensic laboratories. The tool is made freely available to the scientific community at request. Italian and English versions are available.

P30. RAPID SCREENING METHOD COVERS COMMON 161 FORENSIC DRUGS BY ULTRA-HIGH SPEED LC/MS/MS WITH SYNCHRONIZED SURVEY SCANNING

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LC/MS/MS has become an essential tool for the routine analysis in the field of forensic toxicology. It allows you to conduct the simultaneous analysis of multiple compounds in a single run which makes it possible to carry out a fast and high throughput analysis. When ultra-high speed analysis is conducted using UHPLC (Ultra High Performance LC), peaks entering the MS are extremely narrow. However, with conventional MS/MS instruments that cannot handle ultra-high speeds, duty cycles are long, and acquisition rates are slow, resulting in a deficiency of data points across each peak. We developed rapid screening method using ultra-high speed LC/MS/MS with synchronized survey scanning that automatically performs product ion scan (PIS) when a precursor threshold is exceeded in multiple reaction monitoring (MRM).
The aim of this study was the validation of a newly developed rapid screening method for 161 drugs for forensic purposes using ultra-high speed LC/MS/MS with synchronized survey scanning. The use of developed method with synchronized survey scanning enables both qualitative by PIS and quantitative by MRM results in a single run.
Sample preparation was carried out by the simplified QuEChERS extraction method.Subsequently, extracts were measured using a Nexera UHPLC system and LCMS-8040 ultra-high speed LC/MS/MS (Shimadzu Corporation, Japan) operated with synchronized survey scanning. Extracts were separated on a Phenomenex kinetex XB-C18 (100x2mm, 2.6µm) at a column temperature of 40 °C. A flow rate of 0.3 mL/min was used with a binary gradient system and analysis time is only 10 min. 10mM ammonium formate with 0.1% Formic acid in water and 10mM ammonium formate with 0.1% Formic acid in methanol were used for mobile phases. The developed method was registered information (1st coefficient and intersection) of calibration curve for 161 drugs. Semi-quantification was carried out using diazepam-d5 and Phenobarbital-d5 as internal standards.
We evaluated this method couple to modified QuEChERS using standard drugs spiked into human whole blood. The peak area was calculated for each compound and the percentage recovery and matrix effect were confirmed. Percentage recovery was 82.6 % and percentage matrix effect was 94.6 %. Calibration curves constructed in the range from 0.001 to 1 ng/uL for 26 drugs, from 0.01 to 10 ng/uL for 114 drugs and from 0.1 to 100 ng/uL for 21 drugs. All calibration curves displayed linearity with an $R^2 > 0.995$ and excellent reproducibility was observed for all compounds (CV < 10%) at low concentration level. Some different matrices (blood, muscle, liver, etc.) were prepared and 23 drugs were spiked into extract solution. No matrix effect was observed using modified QueChERS extraction. Moreover we confirmed that all drugs were identified from the MS/MS spectra by matching against the highest scoring MS/MS spectra library in terms of degree of similarity.
We developed rapid screening method for 161 drugs for forensic purposes with synchronized survey scanning capable of both qualitative and quantitative results in a single run. The combination of the developed method with ultra-high speed LC/MS/MS and modified QuEChERS enable to strengthen the efficiency of drug screening.

P31. THE USE OF IONIC LIQUIDS FOR THE FAST AND SIMPLE EXTRACTION OF BENZODIAZEPINES FROM COMPLEX BIOLOGICAL SAMPLES

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In forensic toxicology, trace analysis of a wide variety of legal and illegal drugs in complex biological samples is daily routine. Due to the inability of current analytical instruments to directly analyze these complex matrices, a preliminary sample preparation step is still indispensable in the analytical process. To date, various labor-intensive and time-consuming sample preparation techniques have been developed, which all have their specific drawbacks. Moreover, current sample preparation protocols are often a compromise due to strongly varying physicochemical properties within the same class of chemical compounds. For instance, the chemical class of benzodiazepines is characterized by a broad range of pKa values. In this respect, ionic liquids seem to offer new perspectives as alternative extraction solvents. Ionic liquids are liquid salts with good chemical and thermal stability. They have easily adaptable physicochemical features, which is beneficial for the design of task-specific extraction solvents. Overall, ionic liquids seem promising solvents for fast and simple dispersive liquid-liquid micro-extractions (DLLME), eliminating the use of large volumes of conventional toxic solvents.
This study aims at demonstrating the applicability of ionic liquids in the forensic field by developing an ionic liquid-based DLLME procedure for the extraction of benzodiazepines from complex biological matrices such as whole blood.
Preliminary experiments were performed using blank donor whole blood, spiked with 23 benzodiazepines, 2 benzodiazepine-like hypnotics (alprazolam, bromazepam, brotizolam, chlordiazepoxide, chlornordiazepam, clobazam, clonazepam, cloxazolam, diazepam, ethylloflazepate, flunitrazepam, flurazepam, lorazepam, lormetazepam, loprazolam, midazolam, nitrazepam, nordiazepam, oxazepam, prazepam, temazepam, tetrazepam, triazolam, zolpidem, zopiclone) and corresponding deuterated internal standards, at 100 ng/mL. 1 mL of spiked whole blood was transferred into a glass conical tube and extracted using 100 µL of ionic liquid: 1-butyl-3-methylimidazolium hexafluorophosphate. The tube was placed in a rotary mixer for 40 min, cooled in ice for 1 min and centrifuged at 3500 rpm for 12 min. 50 µL of ionic liquid phase was diluted 1/20 with methanol and analyzed using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), equipped with an electrospray ionization source. As mobile

phase, acetonitrile and H_2O pH 9.0 were used in gradient elution. A Thermo Scientific™ Accucore™ column was used for reversed phase separation of compounds. Pre-extraction spiked samples (n=3)(C) were compared to post-extraction spiked samples (n=3)(B) in order to determine recovery (C/B x 100). Repeatability was calculated as the relative standard deviation (%RSD) within a group of three pre-extraction spiked samples. Post-extraction spiked samples (n=3)(B) were compared to standard in methanol (n=3)(A), to determine matrix effects (B/A x 100). Calculations were based on peak area ratios of analyte and internal standard.
Preliminary results showed recoveries within 80-110%, except for bromazepam (62%), lorazepam (131%) and nordiazepam (68%). Extraction repeatability results showed %RSD values < 10%, indicating good repeatability. Matrix effects were within the range of 75-125% (%RSD ≤ 16%). The method will be fully validated and other interesting sample matrices will be analyzed, such as urine and sweat.
Ionic liquids are found to be promising solvents for the extraction of complex forensic samples.

P32. MULTIVARIATE STATISTICAL EVALUATION OF FATTY ACID ETHYL ESTERS (FAEES) COMBINED WITH OTHER BIOLOGICAL MARKERS FOR DIAGNOSIS OF ALCOHOL ABUSE

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An important goal of forensic and clinical toxicology is to identify biological markers of ethanol consumption that allow an objective diagnosis of chronic alcohol misuse. Among indirect biomarkers, the ones most commonly used are aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (Ð-GT), mean corpuscular volume (MCV), and carbohydrate-deficient transferrin (CDT). With respect to indirect biomarkers, direct ethanol metabolites, namely ethyl glucuronide (EtG) and fatty acid ethyl esters (FAEEs), appear to be more specific and sensitive, in particular when they are determined in the keratin matrix. Therefore, their determination currently represents the most accredited strategy for proving chronic alcohol abuse.
The present work focuses on the determination of indirect biomarkers in blood and EtG and FAEEs in hair. Our aims were i) to investigate the diagnostic performances of FAEEs, compared to EtG, and ii) to adopt a chemometric approach to combine the results of both direct and indirect biomarkers in order to provide a more sensitive and specific diagnosis of chronic alcohol abuse.
Blood and hair samples were taken from 157 subjects undergoing through driving re-licensing. Age, gender, BMI, hair color and cosmetic treatments were recorded. Indirect biomarkers were determined in blood and direct biomarkers in hair. Only the proximal segment 0-3 was analyzed. Using EtG as the gold standard, sensitivity and specificity for FAEE were evaluated by means of ROC curves. ROC curves were built using the R Software. Direct and indirect biomarkers were combined through two different multivariate data analysis, namely Principal Component Analysys (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA).
Best ROC curve was obtained by adopting a total FAAEs value of 0.5 pg/mg as cut-off, showing an AUC value equal to 0.86, which in turns corresponds to high sensitivity (96%) but low specificity (76%). Afterwards, PCA and PLS-DA models were developed; the best predictive models were obtained by excluding indirect biomarkers and using FAEEs and EtG only. In particular, PLS-DA approach allowed us to discriminate between chronic and non-chronic alcohol abusers with both specificity and sensitivity equal to 100%.
According to our preliminary results, FAEEs determination appears not to be used alone for assessment of chronic alcohol abuse, because of its low specificity. Nevertheless, its high sensitivity allows i) one to use FAEEs in combination with EtG, in order to corroborate a diagnosis of chronic abuse, and ii) use FAEEs in cases when anomalous EtG values are obtained. The chemometric approach proved highly effective with regard to a correct discrimination between chronic and non-chronic alcohol abusers. Real cases will be presented.

P33. ANALYSIS OF ALKALOIDS OF CHELIDONIUM MAJUS BY THERMAL DESORPTION SURFACE-IONIZATION SPECTROSCOPY METHOD

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Chelidonium majus L. in the last decades more and more attention of researchers more. Containing in a plant alkaloid sangvinarin on pharmacological properties shows the weak psychotropic activity similar to strychnine, causing thus excitement of nervous system, and in high doses causes paralysis of nervous system, strengthens a vermicular movement of intestines and salivation, possesses antimicrobic and fungicide activity. Such alkaloids as helidonin, homohelidonin and metoksihelidonin detain cell fission like colchicine. Helidonin possesses properties close to opium alkaloids, it has antispastic effect.
To develop a technique of the analysis of the helidonin and a sangvinarin from plant raw material by thermal desorption surface-ionization spectroscopy (TDSIS) method.
Alkaloids of a Chelidonium from medicinal plant raw materials isolated by an extraction method. 10 g of samples of dry vegetable raw materials carefully crushed and extracted ethyl oxide in the portions on 10 ml three times. Extracts united and filtered. Added 20 ml of 10% of solution of hydrochloric acid and 5 ml of the cleared water to a filtrate. The water layer was separated, and an organic layer still reekstragirovat with mix 10% of solution of hydrochloric acid and the cleared water (4:1) twice. Added 25% to water extraction ammonia solution to rn=9, extracted chloroform in the portions on 10 ml three times. Chloroformic extract was filtered through 3-5 g of waterless sulfate of sodium. Chloroformic extract of an uparivala at the room temperature dry. The dry rest was dissolved in 2 ml of ethyl alcohol. 1 ÐÐÐ the received spirit

solution entered into a cylindrical cavity of a paroobrazuyushchy tape of the device PII-N-S "Iskovich-1". Then received thermostripping superficial and ionization ranges in the following conditions of the analysis: the emitter – the oxidized molybdenum incorporating iridium; emitter tension – 405 V; emitter-200-250 temperature °C; evaporation temperature – 20-505 °C; an air stream – 50 l/hour (tension of the compressor of 12 V); test volume – 0,1; analysis time – 3 minutes. Previously by means of the series of standard solutions of a helidonin, a sangvinarin having different concentration thermostripping superficial and ionization ranges were received. Using these ranges, the calibration schedule of dependence of current of TDSI of a range of a helidonin on its concentration in solution was constructed. Analyzed the extract received from a chistotel in above the given conditions. Thus observed emergence of peak, characteristic for a helidonin in a temperature interval 189-200 °C, and a sangvinarina in a temperature interval 193-208 °C. Defined the quantitative maintenance of a helidonin and sangvinarin in extract of a chistotel by the calibration schedule.
The developed technique of detection of alkaloids of a chistotel is recommended by method of thermostripping superficial and ionization spectroscopy for application in the quantitative analysis of the helidonin and sangvinarin extracted from vegetable raw materials at poisoning with this plant.

P34. DETERMINATION OF TETRAHYDROCANNABINOL (THC) AND ITS MAIN METABOLITES USING GC TRPLE QUADRUPOLE.

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Tetrahydrocannabinol (THC)is the main psychoactive constituent of cannabis.In most of the countries possession and use of cannabis is illegal, and drugs of abuse tests are implemented to assess the level of consumption. One of the most reliable testing approaches is the employ of a GC coupled to a triple quadrupole mass spectrometer operating in MS/MS mode.
To introduce a novel concept of triple quadrupole: the Thermo Fisher Scientific TSQ Duo is designed to speed up the transition from standalone GC/single quadrupole to triple quadrupole and is capable of showing excellent performances in both the modalities.
The TSQ Duo is hereby operated as a triple quadrupole in MS/MS mode for the analysis of THC and its metabolites THC-OH and THC-COOH in matrixes such as blood and hair.
The instrument can boast excellent linearity and sensitivity in the analysis of samples extracted from both the matrixes.
The TSQ Duo is a versatile instrument capable of working in different modalities. it can employ different workflows to ease the transition from single to triple quadrupole and the methods import and development. Fully controlled by the renown Thermo Fisher Scientific Chromeleon CDS, the TSQ Duo represent a viable option for the analysis of THC and its metabolites.

P35. APPLICATION OF UV-SPECTROPHOTOMETRY METHOD IN THE ANALYSIS OF PIROXICAM ISOLATED FROM BIOLOGICAL OBJECT

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In judicial - chemical practice the cases of acute poisonings with piroxicam are known. In this regard problems of isolation and detection of piroxicam in biological objects are of special interest for carrying out judicial and chemical examination.
Application of UV-spectrophotometry method in the analysis of piroxicam isolated from biological object.
The optical density of 0,01% standard solution of piroxicam in the diluted solution of sodium hydroxide was measured on the spectrophotometer "Cary 60" of Agilent Technologies firm. It is established that piroxicam has absorption maxima 358 nm. For development of a technique for the quantitative analysis of piroxicam by UV-spectrophotometry method the calibration curves of dependence of optical density on concentration of standard solution of piroxicam in sodium hydroxide solution in the field of an absorption maximum. In order to check the suitability of the technique developed by us the amount of the preparation isolated from bioobject was determined. For this purpose the model mixture of biological object containing 50 mg of piroxicam was prepared. 10 g of the crushed biological material was brought in a flask with a capacity of 250-500 ml and filled in with 0,02 mol/l solution of sulfuric acid to a covering of solid particles of biological material. Contents of a flask were mixed and the medium pH was adjusted with 20% solution of sulfuric acid to 2,5. Contents of a flask were left for 2 h at periodic mixing, and then filtered through gauze. Solid particles of biological material were left for an hour with new portions of 0,02 mol/l solution of sulfuric acid twice more, leading pH up to 2,5. Acidic aqueous extracts were connected and centrifuged. Centrifugat was separated and 20-30 ml of 0,02 M solution of sulfuric acid was added to a precipitate and insisted for 2 h. Then it was centrifuged. Obtained centrifugat was separated and added to the first centrifugat. United centrifugat was extracted three times with 30 ml of diethyl ether. Ether extracts was separated and ether was evaporated, the residue was dissolved in the diluted sodium hydroxide solution. The optical density of these solutions was determined on the spectrophotometer at the wavelength of 325 nm. Amount of the piroxicam isolated from biological object was determined by the calibration curves.
It is established that piroxicam solution in the diluted sodium hydroxide solution of has an absorption maximum 358 nm. The detection limit of piroxicam by UV-spectrophotometry method makes 20 μg/ml. Linear-dynamic range of detection lies in the field of concentration of 100-20 μg/ml. Piroxicam was isolated from biological material in amount of 54,3%. Possibility of application of UV-spectrophotometry method in the analysis of the piroxicam isolated from biological object is shown.

Poster abstracts

P36. **USE OF THE HPLC METHOD IN THE ANALYSIS OF PIROXICAM, ISOLATED FROM BIOLOGICAL LIQUIDS**

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In the practice of chemical and toxicological research and judicial and chemical examination the cases of sharp and chronic poisonings with piroxicam became frequent. Study of literature showed that HPLC techniques of analysis of the piroxicam extracted from biological liquids are insufficiently developed. There are no data on researches conducted on validation of a technique and on influence of physical and chemical properties and the nature of objects from which piroxicam is isolated on results of the analysis.
Development of a HPLC technique for the analysis of piroxicam suitable for its determination in chemical - toxicological and judicial - chemical objects.
The liquid chromatograph "Agilent 1100 series" of Agilent Technologies firm with "Chemstation A.09.03" software is used in this work. The device is supplied with the four-channel gradient pump, a degasser and a spectrophotometric UV/VIS detector with variable wavelength from 190 to 600 nm. The analysis was carried out on the column (150 x 3 mm) filled with a sorbent of reverse-phase type of the Zorbax Eclipse XDB C-18 brand with a size of particles of 3,5 microns. Flow rate of a mobile phase - 0,75 ml/min. Researches were conducted in the mode of isocratic eluation. When piroxicam was chromatographed in above mentioned conditions the retention time made 2,29 min. In order to carry out a validation of the developed technique in the above mentioned conditions a series of piroxicam solutions with various concentrations was chromatographed. On the obtained chromatograms and according to the integrator the dependence of the area of chromatographic peak on piroxicam concentration was determined.
It is shown that the developed HPLC technique for determination of piroxicam has rather high sensitivity (0,5 μg). Thus a linearly-dynamic range is in the range of concentration of 0,5-10,0 μg of substance in a sample. On the basis of these data calibration curves of dependence of the area of chromatographic peak on concentration of substance in a sample were drawn for quantitative determination of piroxicam. For checking accuracy and repeatability of the developed technique the working standard solutions containing exact amounts of piroxicam were prepared. Samples of these solutions in volume of 20 μl were consistently entered into a chromatograph injector. Then on the basis of obtained chromatogram the retention time and the amount of piroxicam were determined. Results showed that the relative error of average result of measurements of two studied concentrations are in ranges of 0,92-1,82%. These values show that the developed HPLC technique for determination of piroxicam is rather accurate and repeatable. The HPLC technique for analysis of piroxicam is developed. Such indicators as the accuracy, repeatability, linearly - dynamic range and an error of average result are studied.

P37. **CHEMICAL CHARACTERIZATION AND MICROBIOLOGICAL ANALYSIS FOR QUALITY CONTROL OF METFORMIN DRUG SUBSTANCE**

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Metformin is a potent antidiabetic drug of biguanid class, considered as the first line treatment of diabetes mellitus particularly in overweight obese people and people with normal kidney function.
The objective of this work was to identify and to characterize the active pharmaceutical ingredient metformin and its related substances (impurities) by HPLC and to evaluate its microbiological quality.
Metformin was identified through its organoleptic characteristics, its melting point and by an infrared absorption using Spectrum One FTIR spectrometer. The determination of its purity was performed by potentiometry using perchloric acid. The identification and the dosage of related substances of metformin were carried out using a Perkin Elmer HPLC, equipped with a UV detector at 218 nm and Discovery HS C8 column (250 mm X 4.8 mm X 5 μm) which is maintained at room temperature. A mixture of methanol and ammonium phosphate buffer solution (60: 40) was used as mobile phase; the flow rate was 1 ml / min. Microbiological control was based on enumeration of total viable bacteria and on the search of specified germs « Escherichia Coli ».
The analyzed metformin hydrochloride contains 99.47 per cent of 1,1-Dimethylbiguanide hydrochloride calculated by reference to the dried substance. The analysis of the drug product by HPLC showed four impurities: impurity A (cyanoguanidine), impurity D (1, 3, 5-triazine-2, 4, 6-triamine), impurity E (1-methylbiguanide) and impurity B (4,6-diamino-1,3,5-triazin-2yl)guanidine. the percentage content of each impurity was respectively: 0.0023% 0.0016%, 0.0003% and 0, 0021%. Microbiological analysis of the final product showed that it is free of total viable aerobic bacteria, yeasts, molds and Escherichia coli.
Our drug substance is therefore consistent with the standards required by the pharmacopoeia, reflecting its good chemical and microbiological quality.

P38. **AN AUTOMATED UHPLC-MS/MS METHOD FOR IDENTIFICATION AND QUANTIFICATION OF 10 BENZODIAZEPINES IN ENZYMATICALLY HYDROLYZED DILUTED URINE.**

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Benzodiazepines are normally prescribed against anxiety, as an antidepressant or as a sedative. The effect is mediated by lowering the frequency of breath, pulse and blood pressure. Due to their very addictive nature even at their therapeutic concentrations it is important to monitor misuse of benzodiazepines. Increased sample load and demands of shorter turn-around-times make the need for simplified sample preparation procedures and automation

evident. In this study we used direct injection of enzyme treated and diluted urine samples to quantify 10 common benzodiazepines.
To develop and validate an automated UHPLC method with direct injection of enzymatically hydrolyzed diluted urine according to European Medical Associations guidelines.
Sample preparation was performed on a Biomek® NXP pipetting robot from Beckman Coulter. The analytes were hydrolyzed with β-glucuronidase at room temperature for 20 minutes. 7-aminonitrazepam-d5, 7-aminoclonazepam-d4, 7-aminoflunitrazepam-d7, OH-alprazolam-d5, nordiazepam-d5, oxazepam-d5, temazepam-d5 were used as internal standards. The mass spectrometric analysis was conducted on an Acquity ultra-performance liquid chromatographic system equipped with a Quattro Premier XE™ instrument from Waters operating in positive mode with electrospray ionization of analytes and deuterated internal standards. The chromatographic separation was performed on a HSS T3 RP18 column (2.1 mm x 100 mm, 1.7 µm, Waters). Total injection to injection run time was 5 minutes using gradient elution with 0.1% formic acid as A and methanol as B. Urine samples analyzed in the method application were positive in CEDIA benzodiazepine screening.
The method was validated regarding accuracy, precision, LOD, LLOQ, robustness and matrix effects. The measuring range was 50-5000 ng/mL with a LLOQ between 10-30 ng/mL for all 10 analytes. Precision was below 15% for all analytes at the concentrations; 50, 1000 and 5000 ng/mL. Data from 4275 routine samples analyzed over a period of 10 months showed that 85% were found positive using a cut-off level at 60 ng/mL. Approximately 2000 samples/column were analyzed.
We have developed a fast, sensitive and robust method for the quantification of 10 benzodiazepines in urine which has enabled our laboratory to decrease turn-around-time and minimize work load considerably. The LLOQ for all analytes were lower than the target measuring range to match the screening cut-off level. The automatic reading of barcodes, increased precision and automatic transfer of batch lists to the mass spectrometry software has also greatly increased the quality assurance, altogether making the method an excellent choice for clinical routine analysis.

P39. A NOVEL AND FAST WORKFLOW FOR FORENSIC TOXICOLOGICAL SCREENING AND QUANTITATION USING QTOF LC-MS/MS SYSTEM

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Forensic toxicological screening is challenging in that: (1) the target compound list can exceed hundreds with drastically varying chemical properties, (2) new compounds are constantly introduced; unrecognizable by targeted approaches, (3) common detection techniques (e.g. immunoassay) lack flexibility to adaption to new analytes, and (4) these same techniques often yield significant false-positive and false-negative rates. An alternative technique that is more sensitive and specific is therefore required for efficient screening and quantification.
We aimed to develop a sensitive and selective forensic toxicological screening workflow by utilizing a Q-TOF system and novel MSMS-All with SWATH acquisition.
Blank human urine samples, spiked with more than 50 common drugs, were diluted 10-fold in 10% methanol, centrifuged and 10uL sample was injected. HPLC separation was performed on two different reverse-phase columns (50 × 2.1 and 20 × 2.1 mm) at 30 degree centigrade. Two different LC gradients (6.5-min and 2-min) were developed. Data was collected on a Q-TOF mass spectrometer: 1) TOF-MS survey scan with Information Dependent Acquisition (IDA)-triggering up to 20 product ion scans or 2) SWATH acquisition.
Both the 6.5 and 2.0-min LC gradients using both IDA-MSMS and SWATH acquisition were tested using multiple screening confidence criteria: mass accuracy, RT, isotope and library match. Due to better LC separation and reduced matrix effects, the 6.5-min method yielded better MS/MS library matching score than the 2-min method (87% vs 81% with Purity score, and 96% vs 87% for Fit score). 84% true positive rate with MS/MS matching was observed for 2-min method with IDA-MS/MS which was further improved to 93% using SWATH. Overall the MS/MS mode provided better limit of detection amid higher specificity over TOF-MS. In TOF-MS mode, the quantitation performance was better with 6.5-min LC method, especially for several pairs of drugs that were not easily resolved by the 2-min method, such as 6-MAM/naloxone and norcodeine/norhydrocodone. Because of the shorter runtime and higher flow rate, some drugs (amphetamine, PCP, THC-COOH) showed slightly better sensitivity with 2-min LC method. In MS/MS mode, SWATH acquisition ensures that all data is acquired at all times. Further, variable-SWATH-window approach was used to improve the MS/MS selectivity resulting in sensitive and unambiguous quantitation using fragment ions. Compounds that benefited from the variable SWATH window acquisition included (but were not limited to) the following compounds: 6-MAM, codeine, EDDP, hydrocodone, hydromorphone, MDA, methadone, naloxone, norcodeine and norhydrocodone.
A sensitive and selective workflow was developed for forensic toxicological drug screening using the TripleTOF® 4600 system. Both the IDA-MS/MS and the novel MS/MS with SWATH acquisition were used for screening; both yielding excellent performance. Further, SWATH, which acquired MS/MS data continuously, allows consistent number of data points across the LC peak to be acquired. This not only allows for more confident screening but also for simultaneous quantitation performance.

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P40. THE DEVELOPMENT OF AN LC-MS/MS SCREENING METHOD FOR 104 TARGETED COMPOUNDS IN WHOLE BLOOD, USING LIBRARY SEARCHING ON A QTRAP MASS SPECTROMETER

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In order to detect a large variety of drugs in whole blood, many forensic laboratories incorporate multiple screening assays to cover different drug classes. Each assay screens for a single compound class, and frequently the assay cannot distinguish between specific analytes within a class.
Our objective was to develop a single LC-MS/MS assay capable of accurately identifying >100 target compounds.
Our method employs MRM (Multiple Reaction Monitoring) measurements on an SCIEX 3200 QTRAP LC/MS/MS system to detect 104 target compounds in less than 10 minutes. The QTRAP enabled simultaneous MRM detection and 'on-the-fly' acquisition of a full-scan MS/MS spectrum for every detected compound. All acquired MS/MS spectra were searched against a spectral reference library, to increase confidence in compound identifications compared to traditional MRM-based methods. 500 uL of whole blood containing internal standard was vortex mixed with 3ml of acetone, the sample was centrifuged, and the clean supernatant was collected and dried under nitrogen gas, then reconstituted with MeOH prior to analysis by LC-MS/MS. LC separation was achieved using a Phenomenex Kinetex PFP (50x2.1mm, 2.6um) column.
A cross-method comparison with an outside laboratory demonstrated that our method (i) provided more specific information about compound identity, (ii) provided superior sensitivity, and (iii) detected more compounds. The external testing only detected the presence of a compound class, for example "opiates", whereas the QTRAP screening method identified specific opiate compounds such as Oxycodone, Noroxycodone, Dihydrocodeine, etc. In certain cases the superior sensitivity of the QTRAP screening method detected the presence of compounds that were missed by the external testing. The established cut-off level was 10 ng/mL for the majority of the basic drugs, 1 ng/ml for the fentanyl group and PCP, 250 ng/mL for Trazodone, Pregabalin, and Gabapentin, and 1000 ng/mL for Carisoprodol and Meprobamate.
A comprehensive LC-MS/MS drug screening assay was developed on a QTRAP system that allowed confident identification through library searching. The method has shown to successfully identify compounds in samples that were not detected when analyzed by traditional methods.

P41. THE NEVER ENDING STORY OF CANNABINOIDS IN HAIR

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In hair analysis, the distinction between active Cannabis consumption and external contamination is a known problem. The SoHT recommends the use of cut-offs for THC (50 pg/mg) and its metabolite THC-COOH (0.2 pg/mg) to prove the active consumption. Nevertheless, several studies pointed out that frequently THC-COOH could not be detected in hair, despite considerable THC concentrations were present and highly sensitive analytical methods were applied. Therefore, THCA-A has been proposed as specific marker to prove external contamination. The ratio THC-A/THC has been preliminarily proposed as a discriminating factor.
In this study, we analyzed 78 hair samples (60 head and 18 pubic) previously tested positive for THC, in order to i) evaluate the frequency of THC-COOH positive samples, in comparison to THC positive; ii) evaluate the possible correlation between THC and THC-COOH levels, and iii) evaluate the reliability of ratio THCA-A/THC as a valid marker to discriminate between active consumption and external contamination.
A specific method for the detection of THC, THC-COOH and THC-A was developed and validated. After hair digestion and liquid-liquid extraction with hexane:ethylacetate 9:1, the extracts were injected into a UHPLC system equipped with a C18 column and coupled to a hybrid QqQ-LIT mass spectrometer system. THC and THC-A were determined using MS/MS transitions, while THC-COOH was monitored in MS3.
LODs for THC, THC-COOH and THC-A were, respectively, 5.30, 0.07 and 0.60 pg/mg. Among 78 samples, 30 tested negative for THC-COOH or below LOQ. Among the 48 positive samples (true active consumers), THC-COOH levels were in the range 0.15-8.93 pg/mg (median: 1.40 pg/mg) while THC and THC-COOH concentrations resulted uncorrelated (=0.2238). Among head hair samples, the ratio THCA-A/THC was in the range 0.29-4.95. In these cases, a combined effect of active use and external contamination was likely to account for these THC and THCA-A levels. Among pubic hair, the ratio THCA-A/THC was in the range 0.48-1.55. For these cases, no external contamination is likely to occur and THC and THCA-A levels should be attributed to active use only. Among 30 samples negative (or <LOQ) for THC-COOH, the ratio THCA-A/THC was in the range 0.68-4.94 (median: 1.99).
The metabolite THC-COOH represents the only certain evidence of active Cannabis consumption; therefore toxicological laboratories should -be equipped to determine it. Unfortunately, it has been widely demonstrated that its presence is often not detectable in hair samples from frequent abusers, especially when THC is in the range 0.05-0.10 ng/mg. If THC-COOH is absent, the ratio THCA-A/THC may be used in order to discriminate between active consumption and external contamination. In this perspective, a cut-off value of 1.6 can be proposed. When THCA-A/THC ratio exceeds 1.6, external contamination may be considered prevalent with respect to active use. Otherwise, absence of THC-COOH combined with THCA-A/THC ratios below 1.6, and low THC absolute levels, altogether determine conditions of inconclusive evidences with regard to frequent Cannabis consumers identification. An extended discussion within the scientific community is needed in order to support or contradict this preliminary proposal.

P42. HIGH SENSITIVITY ANALYSIS OF THC-COOH IN HAIR BY USING AN UFMS GCMS TQ IN NEGATIVE CHEMICAL IONIZATION MODE

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Cannabis and its products are assumed for their psychoactive effects. Nowadays, the products of cannabis are the most highly used illicit substances around the world. Consequently the interest in analysis procedures for verification of use and in general for the evaluation of the extent of drug abuse is increasing in clinical and forensic toxicology.
Differently from the analysis of other abused drugs, the analysis of THC and the relevant metabolites in the keratin matrix presents sensitivity problems. The THC and above all the main metabolite 11-nor-D9-tetrahydrocannabinol-9-carboxylic acid (THCCOOH) have low affinity for this matrix and resulting in a very low rate incorporation into hair. The outcome is lower concentrations in hair compared with other drugs. Additionally, THC is present in cannabis smoke and can be incorporated into the hair only by contamination. For this reason, the accurate detection of THC-COOH in hair is required in order to obtain evidence of effective drug consumption and remove any doubt of environmental's contamination.
Hair Samples were analyzed for the evaluation of analytical sensitivity as well as robustness. The determination of picogram or femtogram per milligram of THC-COOH, which indicates an active consumption, requires a very sensitive and selective instrument such as GC/MS/MS in NCI mode. The hair sample, after being washed, was added with deuterated internal standard and hydrolyzed in 1ml of 1M NaOH at 90 ° C. The digested hair was subjected to a LLE basic extraction with n-hexane:ethyl acetate (9:1) for THC isolation. The previous basic remaining solution was acidified with acetate buffer and acetic acid (pH 4) and then THCCOOH was obtained by a LLE extraction using the same mixture. Dried extracts were derivatized with pentafluoropropionic anhydride and hexafluoroisopropanol and analyzed by GC/MS/MS in NCI mode.
To evaluate analytical sensitivity and robustness with hair, an initial acquisition in FASST mode (Scan/MRM simultaneously) was used for general screening and for the evaluation of the time needed for the elution of all the analytes of interest and the matrix. Subsequently, only MRM acquisition mode was used for detection and quantitation of THC-COOH allowing to easily reach the cut-off concentration of 0.05 pg/mg. Validation steps are currently ongoing (linearity, RSD%, LOD, LOQ) and results will be shown on the poster. As first results, the calibration curve of THC-COOH, from 0.05 to 5 pg/mg is showing good correlation coefficients (R^2 > 0.9987) and the method, in addition to show robustness and reproducibility, has good sensitivity with LOD <0.01 pg/mg.
An Ultrafast GC-MS/MS used in NCI (Negative Chemical Ionization) mode allows to accurately quantify the THC-COOH in hair below the SoHT (0.2 pg/mg) and GTFI (0.1 pg/mg) requested LOQ limit ie 0.05 pg/mg.

P43. FULLY AUTOMATED GC/MS DETERMINATION OF THC, CBN AND CBD IN HAIR BY GC/MS

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The determination of Δ9-tetrahydrocannabinol (THC) and two other cannabis related compounds cannabinol (CBN) and cannabidiol (CBD) in hair is an important routine method in forensic laboratories. In Germany, for procedures of regranting driver´s license, a limit of quantitation of 0.02 ng/mg THC is required. Typical methods employ a basic digestion of the hair followed by liquid-liquid extraction, derivatization and GC/MS analysis.
Aim of this study was the comprehensive automation of the determination of THC, CBN and CBD in hair. THC-COOH was not included since the required cut-off (0.2 pg/mg SoHT) is very low and according to literature difficult/impossible to reach with GC/EI-MS. Special methods including for instance derivatization with fluorinated reagents and GC/NCI-MS or GC/NCI-MS/MS detection have to be employed. Normally the analysis of THC-COOH is not required routinely in Germany.
A validated analysis method employing manual sample preparation was used as basis for the automated method. An autosampler equipped with different modules (shaker, centrifuge, evaporator etc.) directly coupled to a GC/MS was employed for the application.
100 mg of ground hair (washed with hexane and acetone and dried before grinding) were manually weighed into a 4 mL vial and the vial was positioned on the autosampler tray. All further steps were running automatically. The hair was digested with sodium hydroxide solution at 85°C under shaking, analytes were extracted with hexane/ethylacetate 9/1 (v/v), phases were separated by centrifugation and an aliquot of the extract was transferred to a clean vial. The extract was evaporated to dryness, reconstituted in silylation reagent and injected into the hot GC inlet for inlet derivatization and transfer to the separation column (DB-5MS 30 m x 0.25 mm x 0.25 µm). Detection was done with a single quadrupole mass spectrometer in single ion monitoring mode.
Comprehensive automation of the analysis method was achieved. The method was validated according to GTFCh guidelines. The limit of quantification for THC was 0.008 ng/mg, the extraction efficiency at 0.02 ng/mg was 99% and the repeatability at 0.02 ng/mg was 4.2%. In order to test for carry-over a blank vial was processed - including the complete sample preparation - after a vial with a hair sample spiked at 3 ng/mg. No analyte peaks were visible in the blank chromatogram. Real case samples were analyzed showing good correlation with results of the manual routine method. The newly developed automated analysis method for THC, CBN and CBD in hair fulfills GTFCh validation guidelines and markedly reduces the workload of laboratory personnel. The quality of analysis data is more independent of the operator in comparison to the manual routine analysis method. The system is quite flexible so that other workflows employing liquid-liquid extraction can be automated as well.

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P44. DEVELOPMENT, OPTIMIZATION AND VALIDATION OF AN ANALYTICAL METHOD FOR THE ANALYSIS OF DRUGS BY GC-MS IN POST MORTEM LIVER SAMPLES USING QUECHERS FOR SAMPLE PREPARATION

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In a number of post mortem situations blood is not available and/or is not analyzable, and therefore alternative samples, such as liver, may be used. However, liver is not usually easy to analyze due to its complex and diverse composition. Therefore, an adequate sample preparation procedure is deemed necessary to obtain the analytes in a suitable form for chromatographic analysis. The most used techniques for this purpose are LLE, SPE and SPME, however these are time consuming and laborious. The QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) approach for extraction, which was initially developed for pesticides isolation from food samples, presents great potential for drug extraction from alternative biological specimens with application in forensic toxicology, since it's a cheap, simple, quick and effective method. This study aimed to develop, optimize and validate a new QuEChERS method coupled to GC-MS for the isolation and concentration of several drugs (amitriptyline, cyamemazine, citalopram, clomipramine, clozapine, fluoxetine, haloperidol, levomepromazine, mirtazapine, olanzapine, sertraline, tiapride, ticlopidine, tramadol, trazodone and venlafaxine) from human liver samples.
The initial step consisted in liver (1 g) homogenization, in a beads homogenizer. Next, a single-phase extraction was performed, by addition of 0.5 mL of deionized water, 0.5 g of $MgSO_4$:$NaCH_3CO_2$ (4:1) and 1.5 mL of 1% acetic acid in acetonitrile to 1 mL of homogenate. After agitation and centrifugation, a dispersive SPE (d-SPE) procedure was performed by adding to the supernatant of the first extraction 25 mg of C18 (octadecyl-modified silica) and 150 mg of $MgSO_4$ to eliminate non-polar matrix interferences and residual water. The samples were agitated and centrifuged, and afterwards the obtained supernatants were evaporated to dryness under a gentle nitrogen stream and re-dissolved in methanol. A 2 µL aliquot was then injected in the GC-MS in the SIM mode.
After method development, the optimal conditions were optimized. The method's detection limits were calculated based on a S/N ratio >3, and ranged between 5 and 100 ng/g (the higher values were obtained for tiapride and fluoxetine, with 100 and 50 ng/g respectively), while recoveries ranged from 50.8 to 61.6%, with RSD < 15%. The matrix effect was evaluated, and ranged from 73 to 110% (except for olanzapine and levomepromazine, which were substantially affected). No false positives were obtained by analysis of blank samples (high selectivity), and no carryover between injections was observed.
This method proved to be simple, fast, cheap and efficient to identify drugs of forensic interest in liver samples, as it also performs a sample clean-up, and this was demonstrated by application in authentic forensic cases. This method offers an alternative for sample preparation that can be easily implemented by laboratories in routine analysis of drugs in liver samples.

P45. COMBINING COMPLIMENTARY ION RATIO AND LIBRARY MATCHING CONFIRMATORY TECHNIQUES IN ONE LC-MS/MS METHOD

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Full scan MS/MS library searching is an effective confirmation technique that allows confidence in compound identifications. On a QTRAP instrument in the same experiment as performing library confirmation workflow, quantification can be performed by using Multiple Reaction Monitoring (MRM) as the survey MS scan for compound detection and triggering the full scan product ion scan in an information dependent acquisition (IDA). In many labs two MRM transitions are monitored and the ion ratio used for confirmation purposes. Quite often interfering compounds will distort the ion ratio, potentially causing a false negative result, which can be avoided by the library searching result. On the other hand, some compounds, namely the barbiturates, don't break apart very well in a tandem MS experiment in which to produce a sufficiently enough specific product ion experiment for library searching.
We aimed to combine the ion ratio with library searching confirmatory techniques as a complimentary workflow but achievable in one injection. The objective was to develop a polarity switching IDA triggered Enhanced Product Ion (EPI) method to allow for high-quality screening of over 100 target analytes in 10 minutes.
The LC-MS/MS method consisted of a Shimadzu Prominence XR and SCIEX 4500QTRAP LC/MS/MS System. LC separation was achieved on a Phenomenex Kinetex C18 (3.0 x 50mm, 2.6 u) column using a 10 minute gradient. The MS method employed an MRM^-/MRM^+–IDA–EPI approach set up to monitor two MRMs for the compounds in negative mode and only one MRM per compound in +ve mode but to trigger a full scan product ion spectrum for every detected compound in positive mode only. The LC-MS/MS technique allows the generation of a chemical fingerprint for a compound through the acquisition of a product ion spectrum. All acquired product ion spectra were searched against a forensic MS/MS mass spectral library for compound identification from the positive mode and the negative mode compounds were confirmed using the traditional ion ratio approach. Urine samples were diluted 10-fold in mobile phase A, centrifuged and 10 uL sample was injected.
In the 10 minute LC run time most critical compound separations were achievable, namely morphine/hydromorphone; methamphetamine/phentermine. Separation was not achieved for Quinine/quinidine and Amobarbital/pentobarbital. The MS method successfully triggered a product ion for all the positive mode targeted analytes; all compounds had library match scores >75%. MRM ratio variability results for the negative mode compounds included a Peak area %CV for all transitions of <18% (n=15) and MRM Ratio of <20%CV for all analytes.
A polarity switching IDA triggered EPI method has been developed which allows for high-quality screening and confident identification of 105 target analytes in 10 minutes.

P46. RAPID SCREENING OF 56 DRUGS OF ABUSE WITH ACCURATE MASS FRAGMENT LIBRARY USING DIRECT ANALYSIS IN REAL TIME (DART) COUPLED TO TIME-OF-FLIGHT MASS SPECTROMETRY (TOF-MS)

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Increase in cases involving drugs of abuse continues to be a challenge for law enforcement agencies, and testing backlogs has been a big burden for crime investigation laboratories. To solve this problem, appropriate methods that can quickly identify the presence of drugs of abuse is highly desirable.
The aim of the present study was to illustrate that the ambient direct analysis in real time (DART) ion source coupled with a time-of-flight (TOF) mass spectrometer could act as a powerful analytical tool for the rapid screening and identification of 56 drugs of abuse. TOF-MS in-source collision induced dissociation (CID) mode was employed to provide informative precursor ($[M+H]^+$ ion and fragment ions for establishing a screening library. With minimal sample preparation, the technique enables instantaneous determination of drugs in small amount of seized samples with confidence rapidly.
The standards and seized samples were prepared in methanol. Dip-it® Samplers were placed in the optimal position for sampling, then the automated rack moved perpendicular to the flow of ionizing gas. All DART spectra were obtained in function switching mode, which allowed multiple measurements including parent ions and fragment ions simultaneously within a single data file. Then the MSTools software was used to detect the 56 drugs of abuse quickly. The criteria for confirming positive results were that in the CID mode, in addition to molecular ion, at least two fragment ions must be detected. A library which included the exact mass as well as the structure of parent ions and fragment ions of 56 drugs of abuse was established, and the pattern of ionization in DART ion source was also analyzed. Then, a standardized screening procedure was developed. The limits of detection (LODs) ranged from 20ng/ml to 5000ng/ml. HoweverÐthere are also some drawbacks in the applied strategy. The two groups of isomers -ephedrine and pseudoephedrine, cannabidiol and THC , couldn't be identified because they had the same fragments in the CID mood. In addition, due to DART-TOFMS lacking separation ability, it can't identify mixtures which consist of too many compounds. After validation, the method was applied to real samples, collecting from forensic cases. As for these real samples, further diluted with methanol must be done to prevent overloading of analyte in the system.
A DART-MS method was developed for the detection of 56 drugs of abuse with minimal sample pre-preparation. With the aid of the library set up by us, the MSTools can identify these drugs of abuse rapidly.

P47. THE DEVELOPMENT OF NEW PSYCHOACTIVE SUBSTANCES IN FRANCE

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Our European neighbours have been involved in the fight against new psychoactive substances (NPS) for several years but the seizures from our police forces had been rare. Only the laboratories of French customs analysed large amounts of NPS. Nevertheless, in the last three years, an increased number of French toxicologists have reported detection of NPS use. On the ground, there is limited possibility of prosecutions because most of the NPS are not scheduled in France at this time. However, because of the problems posed, police officers attempt to make it part of their jurisdiction and seize the substances.
We have monitored the recent NPS seizures to characterise their use in France and to participate in raising the awareness of the increase in seizures of these substances in France which may have an impact on other areas of Europe.
All seizures were analysed according to standard analytical strategy defined in our quality plan. The analysis consisted of screening by ion mobility spectrometry (Ionscan™ 400B, Smiths Detection) and by gas-chromatography/mass-spectrometry (MSD 5975 GC/MSD, Agilent Technologies) after dilution or decoction in an organic solvent: (i) methanol or (ii) acetonitrile or after (iii) derivatisation by Sil-Prep™. Observed mass spectra were compared to the spectral libraries from the ENFSI-DWG and the SWGDRUG. All results relating to NPS were evaluated and substances and their origins were classified.
Between 2013 and 2014, 56 seizures of NPS were analysed in our laboratory: 4 seizures in 2013 and 52 seizures in 2014, showing a dramatic increase in the last year. If synthetic cannabinoids (SC) were the main substances identified (15 different compounds), we also found five different phenylethylamines (ethylphenidate, MDAI, DCMP, camfetamine, fencamfamine), two tryptamines (5-MeO-DALT and αMT), two benzodiazepines (etizolam and diclazepam), a thiophenic analogue of methamphetamine (methiopropamine), a piperazine (mCPP) and dimethylaminoethanol. These seizures came from different areas of France.
All results were registered in our national database from which some government bodies are informed. If all of the substances identified in the analysed seizures were already known, the increasing number of cases objectivises a geographical extension and a supply diversification which may be representative in other parts of Europe. France has not been spared from NPS and seems to be catching up, unfortunately. In France, we are observing a new kind of market with legal and illegal aspects and an evolution of use previously described in some countries. For example, suppliers now often sell pure powdered substance; packagings are simpler than the previous coloured bags and they clearly provide the name and formula of the substance(s). As in many other countries, there are too few tools to prevent this expansion, however, the French government has begun to register the NPS. Ethylphenidate has been registered since March 17th and some synthetic cannabinoid families should follow. Consequently we monitor NPS seizures with the additional aim of monitoring the consequences of these registrations on composition of seized powders and we continue to make magistrates and police-officers aware of the NPS development.

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P48. DEVELOPMENT AND VALIDATION OF A METHOD FOR THE DETERMINATION OF SELECTED OPIATES IN WHOLE BLOOD USING MICROEXTRACTION IN PACKED SORBENT

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Microextraction in packed syringe (MEPS) is a recent sample preparation technique, and is considered a miniaturization of SPE. This approach for sample preparation is very promising, and has been successfully used for the determination of several classes of drugs in urine, saliva or blood. When compared to traditional sample preparation procedures, MEPS reduces sample preparation time (<10 min), organic solvent consumption (< 4mL) and the cost of analysis.
This work describes a new approach based in MEPS to determine simultaneously morphine, codeine and 6-monoacetylmorphine in whole blood samples by high-performance liquid chromatography coupled to coulometric electrochemical detection.
The extraction technique was optimized by means of fractional factorial planning (2k-1), a statistical tool applied to the decision process that assesses in a multivariate way the factors involved. The final optimized conditions were: number of strokes (20), amount of formic acid in the washing solution (3.36%), number of washes of the sorbent (1), amount of ammonium hydroxide in the elution solution (2.36%) and number of elutions (11). Analyses were carried out using an HPLC system (Agilent 1260 Infinity LC) equipped with an ESA coulometric detector (5001A). Chromatographic separation was achieved on a Zorbax 300 SB-C18 column using a gradient mobile phase of 10 mM sodium phosphate (pH 6.6) and acetonitrile at 1.2 mL/min. The optimal potentials used were + 450 mV and + 850 mV. Hydromorphine was used as internal standard.
The optimized method was validated according to internationally accepted guidelines. The studied parameters included selectivity, calibration model and linearity, limit of detection and limit of quantification (LLOQ), precision, accuracy, stability and recovery. The method proved to be linear in the range 25-1000 ng/mL with coefficients of determination higher than 0.99 for all analytes using a small sample volume (250 µL). Intra-and inter-day precision and accuracy were adequate (coefficients of variation ranged from 0.8 to 14% and BIAS were within a ± 14% interval). Recoveries ranged from 6% to 22%. The validated method was applied to the analysis of authentic samples, being an advantageous tool not only within clinical and forensic toxicology, but also in assessing heroin, morphine and/or codeine consumption.
The presented methodology is sensitive for the quantitation of opiates with adequate LLOQ (25 ng/mL) using only 250 µL of whole blood. The use of the fractioned design of experiments showed to be an useful tool for method optimization, reducing experiments, minimizing sample processing time, reagents and laboratory work. This is the first time that MEPS was used for the determination of these compounds in biological fluids.

P49. DEVELOPMENT OF THE FIRST POLYCLONAL ANTIBODY FOR THE DETECTION OF THE SYNTHETIC OPIOID AH-7921 AND ITS MAIN METABOLITE NOR-AH-7921

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AH-7921 ([3,4-dichloro-N-(1-(dimethylamino)cyclohexylmethyl)benzamide], doxylam) is a synthetic opioid analgesic, patented in the mid-seventies. It acts as a selective µ-opioid receptor agonist. In animals it has been shown to be generally equipotent to morphine and showing a high addictive potential. This drug is available for sale on the Internet. The primary route of administration is oral consumption. AH-7921 was identified in synthetic cannabinoid and designer cathinone products sold in Japan. It has been associated with non-fatal intoxications and deaths in some countries. AH-7921 is metabolised to nor AH-7921 and di-nor-AH7921. A first case where AH-7921 was identified by the Division of Forensic Toxicology as the cause of death of an individual has been reported. The availability of efficient immunoassays allowing the detection of this compound and metabolites represents an advantage in the screening stage of the drug testing process.
The aim of the study was to develop the first polyclonal antibody to AH-7921 and its main metabolite Nor-AH-7921. This antibody could be used in the development of sensitive immunoassays for the screening of these compounds in biological fluids.
The immunogen was prepared by conjugating the hapten to a carrier protein Keyhole Limpet Hemocyanin (KLH) using standard methods of conjugation. The immunogen was administered to adult sheep on a monthly basis to provide target-specific polyclonal antiserum. IgG was extracted from the antiserum and evaluated via competitive Enzyme-Linked Immunosorbent Assay (ELISA).
In this initial evaluation the assay was standardised against AH-7921 and the metabolite Nor-AH-7921 was also detected with a cross-reactivity value of 94%. The assay sensitivity, expressed as a half maximal inhibitory concentration (IC_{50}) was 0.081 ng/mL (assay range: 0-10 ng/mL) The intra-assay precision, expressed as CV (%) (n=3), was < 5%.
The results indicate that the developed polyclonal antibody detects the synthetic opioid AH-7921 and the metabolite nor-AH-7921. This antibody is therefore suitable for the development of competitive immunoassays for the detection and quantification of these compounds in human biological fluids for forensic and toxicology applications.

P50. FLUORIDE REGENERATION AND GC-MS WITH LARGE VOLUME INJECTION OF O-ALKYL-METHYLPHOSPHONOFLUORIDATES FOR VERIFYING NERVE GAS EXPOSURE

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Forensic toxicologists are required to identify toxic substances in chemical warfare/terrorism acts such as Tokyo subway sarin gas attack. Nerve gases bind irreversibly to cholinesterases (ChEs) to form adducts. In low level exposure, bound products should be detected after their liberation from adducts (fluoride regeneration). Fluoride regeneration and GC-MS methods have been utilized to this purpose. However, it is difficult to confirm adduct formation because of insufficient detection sensitivity.
We developed a new and highly sensitive fluoride regeneration method coupled to GC-MS for verifying nerve gas exposure in blood contaminated samples.
The method includes incubation of compounds with human serum to reach ChE inhibition; incubation with sodium acetate and potassium fluoride for the regeneration; SPE to extract regenerated products; GC-MS detection with large volume injection. O-Ethyl methylphosphonofluoridate, O-isopropyl methylphosphonofluoridate (GB), O-isobutyl methylphosphonofluoridate, O-pinacolyl methylphosphonofluoridate (GD), O-cyclohexyl methylphosphonofluoridate (GF), O-ethyl S-N,N-diisopropylaminoethyl methylphosphonothiorate (VX), O-isobutyl S-N,N-diethylaminoethyl methylphosphonothiorate (RVX) were synthesized in house. GB, GD, GF, VX or RVX was incubated with control human serum (Cosmo Bio Co.) at 25°C for 15 min to reach ChE inhibition to prepare the inhibited serum samples. Fluoride regeneration was performed by incubating the inhibited serum (1 mL) with 0.2 M sodium acetate (pH 3.5, 0.19 mL) and 5.25 M potassium fluoride (3 mL) at 25°C for 15 min. After neutralization, the mixture was applied to BondElute NEXUS cartridge, and regenerated. O-alkyl-methylphosphonofluoridates were eluted with ethyl acetate (2 mL), and an aliquot of 0.5 mL was subjected to GC-MS (AiSTI SCIENCE large-volume injector -Agilent 7890 GC-5977 MSD) with DB-5MS column, in electron ionization mode and full scan (m/z 40-300) acquisition. Nerve gas adulterated sera were assayed for ChE activity using butyrylthiocholine substrate by a colorimetric method using 5,5'-dithio-bis(2-nitrobenzoic acid)
All the nerve gases under examination inhibited serum ChE activity stoichiometrically, indicating ChE active site concentration of 90 nM in the serum. From the fluoride regeneration treatment of the serum inhibited by GB, GD, GF, VX or RVX, the corresponding O-alkyl methylphosphonofluoridates were produced. The injection in GC-MS allowed to separate and detect the target analytes using the ionic species at m/z 99 for reconstructed ion chromatograms. The recoveries of the fluoride regeneration were around 60% with RSD less than 7% at 10 ng/mL. except for GD whose recovery was lower than 10%. The linear range in the calibration plot was from 2 to 10 ng/mL serum. The lower limit of quantification of 0.6 ng/mL nerve gases could be reached for all target analytes in the inhibited serum samples.
Fluoride regeneration, SPE extraction and GC-MS with large volume injection were useful for verifying exposure to 5 nerve gases, through detection of O-alkyl methylphosphonofluoridates from spiked serum samples, with a limit of quantification of 0.6 ng/mL for all analytes except for GD.

P51. A RAPID HEAD SPACE-GAS CHROMATOGRAPHY MASS SPECTROMETRY METHOD FOR DETERMINATION OF CYANIDE IN BLOOD SAMPLES

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Toxicity of cyanide has been well-documented in the literature. Exposure to cyanide results in adverse health effects in both humans and aquatic biota. People can be exposed to cyanide by variety of ways. Cyanide exhibits its toxic action by binding to the heme-type iron complex in cytochrome oxidase, causing an inhibition in the last step in oxidative phosphorylation in the cycle. Cyanide can also affects the structures many proteins and metalloenzymes. Therefore, determination of cyanide in human biological fluids, especially in blood, is of great importance in forensic identification.
We aimed to develop a fast, and reliable sample preparation procedure prior to head space-gas chromatography mass spectrometry analysis for the determination of cyanide concentration in blood samples.
Firstly, a 200 µL of 1 M H_2SO_4 solution was added into a low volume vial insert. This insert containing acid solution was gently placed to bottom of a 22 mL gas chromatography vial. Thereafter, 200 µL of blood sample (or standard solution) were added to dry part of GC vial. After the vial sealed with gas-tight polytetrafluoroethylen (PTFE)-lined rubber septum cap, it was vortexed for 30 seconds. The pretreated sample was then analyzed by HS-GC-MS. The HS-GC-MS conditions were as follows: The headspace oven, needle, and transfer line temperatures were set at 90 °C, 130 °C, and 130 °C, respectively. The injection time was 0.12 min and vial was pressurized to 30 psi in 1.0 min. A Perkin Elmer Elite FFAP (Crossbond Carbowax-PEG for acidic compounds, 30 m × 0.25 mm i.d.) capillary column was utilized. Helium was used as carrier gas. The GC oven temperature was initially set at 40 °C and held for 8 min, the temperature was increased to 140 °C at a rate of 10 °C/min which was held for 3 min, and then elevated to 240 °C at a rate of 30 °C/min which was held for 3 min. The temperatures of Electron Impact ion source and GC line were 200 °C.
Repeatability for the peak area and retention time, selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), and recovery parameters were used for the validation of the analytical method. We obtained excellent RSD (%) values from peak area and retention time of 50 µg/mL cyanide standard solution which were 1.7 and 0.11 (N=10), respectively. Linearity was evaluated by linear regression (R^2=0.999). LOD and LOQ were 0.5 µg/mL and 1.5

µg/mL which were calculated from the peak height as the average concentration corresponding to the signal-to-noise ratio equal to 3 and 10, respectively. Even though acceptable recovery percentage range is 70–120% with a precision about 20% depending on sample matrix in analytical studies, we obtained perfect recovery values which were 100.5 % for a standard solution and 97.9 % for a blood sample spiked with 50 µg/mL cyanide.
In this study, we propose a sample preparation method for headspace GC-MS analysis of cyanide in blood samples. The advantages of the method are easy, fast (max 5 min) and reliable.

P52. GAS CHROMATOGRAPHY/MASS SPECTROMETRY METHOD FOR THE SIMULTANEOUS DETERMINATION OF 9 LOCAL ANESTHETIC DRUGS AND ITS INTERNAL STANDARD (LIDOCAINE-D10) IN HUMAN WHOLE BLOOD, URINE AND SPUTUM

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Local anesthetic drugs are used intravenously, epidurally, spinally, topically or subcutaneously to relieve pain without producing unconsciousness. These drugs are potentially toxic affecting the heart and the central nervous system, if they are administered in high doses or into the wrong anatomic site.
The aims of the study were the development and the validation of a gas chromatography/mass spectrometry (GC/MS) method for the simultaneous identification and quantification of 9 local anesthetic drugs (Lidocaine, Procaine, Mepivacaine, Ropivacaine, Tetracaine, Bupivacaine, T-caine, Oxybuprocaine, Dibucaine) in human whole blood, urine and sputum.
For sample preparation, whole blood (0.5ml), urine (0.5ml) or sputum (0.1ml) was mixed with an equal volume of 0.1% formic acid aqueous solution and 100 µg of cholinesterase inhibitor, such as neostigmine bromide. Extraction was performed with a fivefold volume of tert-butylethyl ether. The resulting mixture was vortexed for 1 min and centrifuged for 5 min at 3000 rpm. The supernatant was transferred into a test tube. 20 % Na2CO3 solution and same volume of tert-butylethyl ether were added to remaining aqueous layer. The resulting mixture was vortexed and centrifuged. The supernatant was transferred into the test tube, again. The organic solvent was evaporated to dryness at room temperature with a stream of nitrogen gas. The residue was dissolved in 100 µl ethyl acetate and transferred into an autosampler vial. GC/MS was performed on an Agilent Technologies 7890A GC System/ Agilent Technologies 5975C inert MSD. The column was an Agilent HP-5MS (30 m × 0.25 mm i.d. 0.25 µm). Preliminary mass spectra of these compounds were obtained in SCAN mode after electron ionization (EI) from m/z 50 to 350, while selective ion monitoring (SIM) mode was used for the quantification.Target compounds were measured based on SIM mode on the following ions; m/z=86 for Lidocaine, m/z=96 for Lidocaine-d10, m/z=86 for Procaine, m/z=98 for Mepivacaine, m/z=126 for Ropivacaine, m/z=58 for Tetracaine, m/z=84,140 for Bupivacaine, m/z=86,176 for T-caine, m/z=192 for Oxybuprocaine and m/z=86 for Dibucaine.
Validation was performed working on spiked human whole blood, urine and sputum. Extraction efficiency and matrix effects were evaluated by analysis of incurred samples. Liquid-liquid extraction recovery of local anesthetics was 76-99%. The limits of detection (LOD) and quantification (LOQ) ranged from 5ng/ml to 70ng/ml and from 10ng/ml to 250ng/ml, respectively. The calibration curve was linear ($R^2 > 0.99$).
The presently established GC/MS method for simultaneous measurements of local anesthetics in human whole blood, urine and sputum is very useful.

P53. LC-MS/MS SCREENING OF ILLICIT AND MEDICINAL DRUGS ON DRIED BLOOD SPOTS

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Dried blood spot (DBS) analysis is an emerging microsampling technique for bioanalysis to support traditional liquid blood analyses studies. It offers many advantages over conventional sample collection methods including much smaller sample volumes, easier collection, easier handling, and importantly cheaper and easier transporting. It has also been reported that DBS can offer advantages in reducing loss of unstable compounds.
The aim of the present study was the validation of an existing validated LC-MS/MS method for the detection and quantification of 126 drugs on dried blood spots. The method covers a broad range of common drugs of abuse including amphetamines, benzodiazepines, cocaine (including metabolites), opioids, new psychoactive drugs and common prescription drugs.
DBS were produced from 10 µL of whole blood and extracted as the existing validated method using butyl chloride as extract and liquid chromatographic separation with an Eclipse XBD C18 separation column, QTrap 5000 mass spectrometer performed with MRM (2 transitions) in positive mode by means of electrospray ionisation.
The method showed good detection for the majority of substances included in the method. The stability of the drugs on DBS was determined over a 1-3 month period stored at different conditions (room temperature, 4Đ and -20Đ). Furthermore, the method was applied to authentic samples and results were compared with those obtained from whole blood.
The ability to detect drugs stored on dried blood spots has been assessed. DBS is a suitable alternative for the determination of many drugs of interest in forensic cases. DBS would be useful in facilitating the process where larger volumes of liquid blood are normally collected and transportation may delay the start of testing.

P54. **A COMPREHENSIVE SCREENING APPROACH TO DETECT TOXICOLOGICAL COMPOUNDS IN HUMAN MATRICES IN FORENSIC CASES.**

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Systematic Toxicological Analysis (STA) is generally brought about by using GC or LC, coupled to mass (MSn) spectrometry. Targeted or non-targeted data acquisition may be used.
To develop a comprehensive screening approach with a state-of-the-art analytical method that determines all relevant toxicological compounds-of-interest in whole blood.
First, we developed a strategy to compile a list of relevant toxicological substances that may be encountered in forensic cases. Secondly, we developed and validated an UPLC-ToF-MS (1290 Infinity/6230A ToF, Agilent) analysis for whole blood. Sample preparation was done by protein precipitation using a mixture of acetonitrile, ethanol and formic acid. Chromatographic separation was carried out with gradient elution at pH 3.5 (Acquity HSS T3 column) and pH 9.2 (Acquity BEH C18 column), followed by +/- ESI. ToF-MS data acquisition was done in 2 GHZ extended dynamic range mode 75-1100 m/z. Validation parameters included robustness of retention time (within 0.15 min of self-compiled database), selectivity (n=20 whole blood), intermediate precision (<30%) and identification score (>70%). Identification score was based on the monoisotopic mass (±10 ppm), the isotope pattern (±7.5%) and the isotope deviation (±2 mDa + 5.6 ppm). Validation was done using eight runs spanning a month period. If validation criteria were met the compound was deemed detected.
We compiled a list of 390 toxicologically relevant compounds including (metabolites of) drugs, medicines and pesticides. Inclusion criteria were: prevalence on the Dutch market, prevalence in earlier forensic cases and in cases from the Dutch poison center, ability to affect driving performance (category II and III ICADTS), possible use in drug-facilitated-sexual-assault (DFSA SOFT list) and acute toxicity. The UPLC-ToF-MS method was validated for all 390 compounds in whole blood at sub therapeutic levels (50% or 25% of the lowest active level with a minimum of 0.0001 mg/l) or at 0.01 mg/l (i.e. for pesticides without reference concentration) and at 10 times that level. Approximately 87% of the compounds (339/390) met the criteria for validation at the lowest and/or 10 times higher level in blood. Most of the compounds (329/339) were detected at pH 3.5 (305 +ESI/73 –ESI), whereas 87% of the compounds (296/339) were detected at pH 9.2 (285 +ESI/37 –ESI). Forty-one compounds were detected at pH 3.5 that were not detected at pH 9.2, whereas 10 compounds were only detected at pH 9.2. Most compounds (319/339) were detected with positive ionization, but for 19 compounds negative ionization was needed. Depending on the forensic importance and prevalence of the missed compounds, a decision can be made on the number of analytical runs needed.
A comprehensive approach was developed to screen for relevant toxicological compounds in whole blood using a sensitive and selective UPLC-ToF-MS method. Decisions on the inclusion of compounds and the evaluation of the method were facilitated by clear criteria and a systematic approach.

P55. **SCREENING FOR CHEMICAL (WARFARE) AGENTS IN HUMAN MATRICES**

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Forensic Toxicologists are part of the CBRN-team within the Netherlands Forensic Institute (NFI). The CBRN-team consists of forensic experts with Chemical, Biological, Radiological and/or Nuclear (CBRN) expertise. The main goal of this team is to optimally perform forensic investigation in case of a possible terrorist attack or threat with a CBRN-agent.
The aim is a screening strategy to determine a possible exposure to an effective or toxic dose of a chemical agent with a broad and specific screening method(s).
First, a list was compiled of relevant (high risk) chemical substances (and/or metabolites) which could be encountered in terrorist attacks. Secondly, due to diversity of the chemical substances four different analytical methods were developed of which two are validated. A targeted screening in whole blood and urine with UPLC-LTQ-Orbitrap MS was validated for the parameters robustness of retention time (within 0.10 min), selectivity (n=10), mass accuracy (within 5ppm deviation from theoretical mass) and precision (<30%). The sample preparation was done by precipitation with acetone followed by evaporation and reconstitution in methanol/water (20:80, v/v). Furthermore, an elemental screening and (semi)quantification method was validated (i.e. LOD, accuracy (80-120%), precision (<20%)) using ICP-MS and ICP-OES. The bloodsample was destruated before the analysis by heating(90°C) subsequently with nitric acid and hydrogen peroxide. A specific HSGC-MS was developed for the quantification of cyanide, sulfide and azide in whole blood after acidification with phosphoric acid. And finally, a complementary screening method organic compounds with GC-QTOF-MS is currently being developed.
The compiled list of high risk compounds contained organic compounds (such as chemical warfare agents, pesticides, and toxic industrial chemicals), elements (such as arsine and mercury) and anionic compounds (such as cyanide and sulfide). Forty-three (metabolites of) the high risk organic compounds in whole blood and 35 in urine met the validation criteria and could be detected with UPLC-LTQ-Orbitrap MS in blood and urine in concentrations varying between 2,5 µg/l and 250 µg/l. The element screening with ICP-MS and ICP-OES met the stated validation criteria and 65 elements could be detected and (semi) quantified in concentrations of 5 µg/l or higher in blood.
A HSGC-MS method was developed to quantify cyanide in whole blood from 0,2 mg/l to 5 mg/l and to simultaneously detect sulfide and azide in concentrations respectively from 0,5 mg/l and 2 mg/l. This method is currently used in a procedure designed for infrequent preformed analysis. This procedure contains extra quality control measures.

Poster abstracts

And finally, a complementary screening method to detect volatile organic compounds with GC-QTOF-MS is currently being developed for over 200 substances in whole blood and urine. Up to now no forensic (postmortem) terroristic case occurred to show the benefits of this developed comprehensive screening approach. However, in non-terroristic forensic (postmortem) cases the developed methods were used separately depending on the question involved. The combination of the established methods is a promising generic screening instrument to identify chemical agents in whole blood and/or urine.

P56. DEVELOPMENT OF MULTI-TARGETED SCREENING METHOD FOR 56 NATURAL TOXINS IN PLASMA BY LC/Q-TOFMS

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There are various wild lives with natural toxins (NTs), which are very important analytical targets in forensic toxicology. However, the wide range of NT properties poses difficulty in attributing cause of death to NTs in routine toxicological analysis, especially since there is currently no effective routine screening method of NT available.
The aim of the present study was to get some kinds of data for developing a rapid and sensitive multi-targeted screening method for 56 NTs in plasma by liquid chromatography/quadrupole-time of flight mass spectrometry (LC/Q-TOFMS).
Target NTs were selected on the basis of previously reported poisoning cases as follows: 9 mushroom toxins, 5 marine toxins, 35 plant toxins, 3 venoms and 4 mycotoxins. All samples were prepared in methanol (MeOH) solution with the exception of some aconitines, which were dissolved in acetonitrile (MeCN). Stock solutions were stored at -30°C until analysis. AB Sciex TripleTOF 5600 mass spectrometer and Shimadzu NexeraX2 LC system were used for analysis. L-column ODS (Chemicals Evaluation and Research Institute, Japan, 1.5×150 mm; particle size, 5 μm) was used for LC separation, and 10 mM aqueous ammonium formate solution and methanol were used as mobile phases with gradient elution (methanol concentration: 5-95% for 15 min). Electrospray ionization was used in both positive and negative modes. Information dependent acquisition (IDA) criteria were set at over 50 cps. For sample preparation, the five extraction methods, which were deproteinization with MeCN, deproteinization with MeOH, deproteinization with MeCN/MeOH mixture, QuEChERS method (AOAC method), and deproteinization filter Captiva ND Lipids (Agilent Technologies), were compared over their sample recovery rates. All target compounds were investigated on isotopic ratio, high resolution product ion spectra at four different collision energies (20, 35, 50 eV and collision energy spread mode), and retention property for qualification by Q-TOFMS or LC/Q-TOFMS.
The combined use of high resolution mass spectrometry and IDA was extremely effective to simultaneously detect NTs in forensic samples. All NT compounds could be extracted by deproteinization with MeCN. However, some compounds showed about one and a half to ten times better recovery rates by deproteinization with MeOH or deproteinization with MeCN/MeOH mixture than deproteinization with MeCN. QuEChERS method showed good sample clean-up effect and Captiva ND Lipids was advantageous for its handling simplicity. Neither method showed better recovery rates compared to deproteinization.
This screening method is thought to be effective for NT screening and to become a powerful tool to search NTs in routine forensic toxicological analysis with simple operations.

P57. INFLUENCE OF THE SAMPLE PREPARATION FOR THE DETERMINATION OF THC AND THE METABOLITES THC-OH AND THC-COOH IN HAIR SAMPLES

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According to the Society of Hair Testing (SoHT) guidelines and the German assessment criteria for drug analysis in cases for the reinstatement of driver licenses, the cannabis agent tetrahydrocannabinol (THC) is used as the target compound in hair analysis for the testing of cannabis consumption. It is known, that when cannabis is consumed, THC is mainly absorbed into the head hair during exposure to side stream smoke. Because of this, it is possible that there are positive THC findings in the lower concentration ranges by people who did not consume cannabis but was in presence of cannabis smoke. Detection of the metabolites, which are built in the liver, can be used to prove a passage through the body.
The aim of the study was to test the influence of two different sample preparation methods for the analysis of THC and the metabolites 11-hydroxy-tetrahydrocannabinol (THC-OH) and 11-nor-carboxy-tetrahydrocannabinol (THC-COOH). Also, to prove the usability of THC-OH analysis as a consumption marker.
THC is an analyte in our multi component screening for drugs of abuse. The hair samples are washed in bulk with petroleum ether followed by a second wash with methanol. Next, the hair is cut into small pieces and extraction performed by ultrasonification with methanol, the extract is then evaporated to dryness and measured with LC-MS^2. Because THC-COOH cannot be extracted with methanol from hair, a separate sample preparation for the determination of THC-COOH is necessary. The hair is dissolved with NaOH followed by a LLE clean-up step with hexane/ethyl acetate and finally SPE. After extraction the samples are evaporated to dryness, derivatisied with iodomethane and measured with LC-MS^2. The LOQ was 0.1 pg/mg. There are two possibilities to test for THC-OH, either in the methanolic extract (from the screening method) or in the hexane / ethyl acetate supernatant from the THC-COOH determination. The both extracts are evaporated to dryness and derivatisied with picolinic acid. After derivatisation the measurement in LC-MS^3 mode is performed with a LOQ of 0.05 pg/mg. It is also possible to determine THC with this method.

The levels of THC and THC-OH in the methanolic hair extract (0.1 – 1.9 ng/mg for THC and 0.05 – 1.5 pg/mg THC-OH) were lower than those measured in the hexane / ethyl acetate phase after hydrolysis (0.1 – 3.1 ng/mg for THC and 0.05 – 15 pg/mg for THC-OH). 374 authentic hair samples were compared in a positive/negative classification. 57% of cases tested positive for all three analytes. In 11% of the cases THC and only one metabolite tested positive. In 10% of the cases only THC tested positive, therefore active consumption could not be proven. In 5% of the cases no THC was detected but at least one of the metabolites.
The results indicate that for proof of active cannabis consumption hair should be tested for the THC metabolites THC-OH and THC-COOH. It was also possible to show that the analysis for THC and its metabolites should be done in one assay after hydrolysis of the hair samples

P58. BEHAVIOR OF HYGRINE AND CUSCOHYGRINE IN ILLICIT COCAINE PRODUCTION ESTABLISHES THEIR USE AS MARKERS FOR DISCRIMINATION BETWEEN CHEWING OF COCA LEAVES AND COCAINE ABUSE

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Hygrine and cuscohygrine are two alkaloids of coca leaves that are proposed as good markers in urine and hair for differentiation of the traditional and legal use of coca leaves for chewing in Argentina vs. the illegal use of cocaine. Their absence in street cocaine seizures can be explained by the loss of some coca alkaloids during the illegal processes of extraction and purification of cocaine from the leaves.
In this work, it was investigated at which step of cocaine production hygrine and cuscohygrine are lost.
The production of illicit cocaine can be achieved in a number of ways. We followed the technique outlined in the Cocaine Manual UNODC. The fresh and dried coca leaves (A) were crushed, mixed with water, lime and kerosene and stirred for 2h. Then, the kerosene is separated from the extracted coca leaves (B) and back-extracted with diluted sulfuric acid in water. At this point the kerosene is discarded and the aqueous layer is made alkaline with ammonia, which results in precipitation of crude cocaine together with the more basic alkaloids. The product is then filtered and dried to give coca paste (C). For analysis, aliquots of (A), (B) and (C) were extracted with tert-butyl methyl ether (TBME) or by a mixture of methanol/acetonitrile/2 mM ammonium formate and analyzed by GC-MS and LC-MS/MS under the same conditions reported in previous work for cocaine (COC), ecgonine methyl ester (EME), cinnamoylcocaine (CIN), tropacocaine (TRO), cuscohygrine (CUS) and hygrine (HYG) .
After extraction with kerosene HYG, CUS as well as EME remain almost completely in the residual coca leaves (B) whereas COC and CIN are found in the coca paste (C). This can be explained by the much higher solubility of COC in the non-polar kerosene in comparison to HYG, CUS and EME as it can also be concluded from the partition coefficient between n-octanol and water log Pow which is 3.08±0.38 for cocaine and only 0.28±0.27, 0.72±0.35 and 0.23±0.37 for HYG, CUS and EME.
The loss of HYG and CUS in the first steps of the illegal cocaine production is essential for using them as markers in order to distinguish chewing coca leaves from the different kinds of illegal cocaine consumption, for instance from smoking coca paste that is frequent in Latin-American.

P59. MEASUREMENT UNCERTAINTY IN DETERMINATION OF AMPHETAMINES IN URINE BY LIQUID-PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Amphetamine-type stimulants refer to a group of drugs which includes as their main substances both amphetamine and methamphetamine. They represent some of the most sought substances in our laboratory premises. The use of amphetamines is still an issue in many countries as it is fast growing and unlikely to decrease in the near future. To sum up, the present situation demands an immediate action, with major focus on reliable analytical methods capable of detecting such compounds in biological fluids. Furthermore, the measurement uncertainty (MU) has been increasingly required in analytical toxicology by quality management standards, such as ISO 17025, to demonstrate the reliability of analytical data and it is needed as a validation parameter. Most laboratories take on decisions based on analytical data, meaning that having a small and reliable MU reflects the accuracy of the method used.
The purpose of this study was to estimate the MU of confirmatory toxicological analysis of amphetamines in urine by liquid-phase microextraction technique (LPME) which were then submitted to analysis through gas chromatography-mass spectrometry (GC-MS).
The pH of urine samples (1 mL) was adjusted using NaOH and the content was then transferred to an Eppendorf tube containing NaCl. The hollow fibers were submitted to contact with di-n-hexyl ether with its lumen filled with HCl and further immersion into urine sample solution. The system was then submitted to agitation. The acceptor phase was withdrawn from the fiber and dried under N2 stream. The residue was then submitted to derivatization with trifluoroacetic anhydride and ethyl acetate. An aliquot of 1.0 µL was then injected into the GC-MS system for analysis. The method was validated and the MU was calculated following the Guide to the Expression of Uncertainty in Measurement.
The limit of detection was 10 ng/mL for amphetamine and 20 ng/mL for metamphetamine. The calibration curves were linear over the specified range (20 ng/mL to 1400 ng/mL; R^2>0.99). The intra-day and inter-day precisions, in the lower concentration levels, were always less than 20% as relative standard deviation and the accuracy was satisfactory (values above 88%). After optimization of the extraction, the average recovery value was 64%. Once

the complete validation has taken place it was applied to authentic human urine samples (n=10) and uncertainty values were calculated for these, considering that only amphetamine has been found. In order of importance, the factors which were more determinant for the calculation of method uncertainty were: analyte concentration, sample volume, accuracy and method precision. Combined uncertainty values were encountered in the range of 2.1% and 5.2%.
LPME has revealed to be an efficient microextraction technique considering it comprises of several advantages such as its low cost, small amount of sample required, easy clean-up and extremely low volume of hazardous solvents. This study has established the MU for the determination of amphetamines in urine samples using LMPE followed by GC-MS. Moreover, combined uncertainty was acceptable, supporting the successful application of the proposed analytical method as it has shown to be both relevant and reliable.

P60. METHODS FOR THE DETECTION OF FETAL ALCOHOL EXPOSURE

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Alcoholic beverages use during pregnancy has been associated with an increased risk of stillbirth. Women who consumed ≥5 drinks/week had a 2–3 times increased risk of experiencing a stillbirth, mainly because of fetoplacental dysfunction. Then, the detection of fetal alcohol exposure is important to elucidate the cause of death. This detection can be performed by maternal drinking history or by biomarkers of alcohol use. Maternal self-reports of gestational drinking are often unreliable due to feelings of embarrassment, fear and guilt. Consequently, laboratory testing to provide indicators of alcohol exposure has great importance. In recent years, fatty acid ethyl esters (FAEEs) in meconium have been reported to be potential biomarkers of fetal exposure to alcohol. In this study, we used four FAEEs (ethyl linoleate, ethyl palmitate, ethyl stearate and ethyl oleate) that have been more commonly associated with fetal alcohol exposure.
To determine the fetal alcohol exposure by screening instruments, interviews and biomarkers in meconium.
Data collection was performed between January 2014 and May 2014. After delivery, data were collected from 160 hospitalized women from a public low-risk obstetric unit in the city of Ribeirão Preto, Brazil. The questionnaires T-ACE and AUDIT were employed as screening instruments for alcohol use. The participants were interviewed about the quantity and frequency of alcohol consumption during first, second and third trimester of pregnancy. The FAEEs were extracted by liquid-liquid extraction followed by solid phase extraction and analyzed by gas chromatography coupled to mass spectrometry, operated in electron impact mode.
50 (31.25%) participants were T-ACE positives (cut-off = 2), that is, alcohol intake potentially sufficient to damage the fetus. 34 (21.25%) participants were AUDIT positive (cut-off = 8) that means indicators of hazardous and harmful alcohol use, as well as possible alcohol dependence. By interview, the frequencies of women who drank five or more standard drinks/week (5x12 grams of alcohol) in the first, second and third gestational trimester were 14.4%, 5.0% and 3.8%, respectively. Meconium analysis showed that the FAEEs could be detected in alcohol-exposed newborns. Ethyl linoleate was the most prevalent FAEE found in positive meconium samples and was detected at the highest levels. FAEEs could also be detected in non-exposed newborns, but the levels were much lower than those measured in positive cases.
The "drinking risk" in this community was 31.25%. The collected data by questionnaires were in agreement with interview information. The developed analytical method was able to detect FAEEs in meconium from live born babies. And we suggest the application this method for meconium analysis in fetal autopsy after stillbirth.

P61. DETERMINATION OF BARBITURATES IN HAIR MATRIX BY LIQUID PHASE MICROEXTRACTION (LPME) AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

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Barbiturates are popular pharmaceutical drugs used as an anti-epileptic and general anesthesia induction. Cases of poisoning are often reported not only by deaths from accidental ingestion and suicide but also as drug-facilitated of crime. For this purpose, pharmaceutical analysis of hair can be a useful tool for drug monitoring, drug compliance, and forensic toxicology in the establishment of a personal drug history or when classical matrices are not available. The technique of choice was LPME in conjunction with GC-MS due to LPME be low cost, convenience and reduced use of organic solvents.
The aim of the present study is the development and application of a liquid phase microextraction (LPME) and gas chromatography–mass spectrometry (GC-MS) method for the simultaneous identification and quantification of the barbiturates most commonly found in Brazil as phenobarbital, secobarbital and pentobarbital, in 50 mg of hair sample.
Drug-free hair specimens were collected and separated in 50 mg aliquots. Each aliquot were washed with 2 mL of dichloromethane for 15 min at 37°C. Phenobarbital, secobarbital, pentobarbital and their respective deuterated were spiked into the same sample and submitted to digestion with sodium hydroxide (NaOH) 1M for 15 min at 70°C. The completely dissolved samples were centrifuged and submitted to LPME. After derivatization with tetramethylammonium hydroxide (TMAH), the samples were applied to the GC-MS.
The limit of quantification (LOQ) was 0,1 ng/mg for all analytes. The calibration curves were linear over a concentration range of 0,1 ng/mg to 10 ng/mg (R^2 = 0,99). The method showed to be precise and accurate (RSD <15%), including dilution integrity tests. The method was applied to two real samples and the concentrations found were

of 9,46 and 16,33 ng/mg of phenobarbital. Due to the lack of commercialization of analytical patterns for barbiturates metabolites and non-predefined cut off values, the analysis of the wash residue were performed to confirm consumption and to exclude contamination.
The development and application of LPME followed by GC-MS is a promising method for the simultaneous identification and quantification of phenobarbital, secobarbital and pentobarbital in hair samples. The procedure used for digestion and extraction of analytes from hair enabled their detection with values of LOQ, precision and bias acceptable within the optimum range.

P62. COLLISION-INDUCED DISSOCIATION PATHWAYS OF HALLUCINOGENIC PHENETHYLAMINES (2C-X) AND THEIR N-(2-METHOXYBENZYL) DERIVATIVES (NBOME) USING HIGH-RESOLUTION MASS SPECTROMETRY FOR NON-TARGETED SCREENING PURPOSES

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Hallucinogenic 2,5-dimethoxyphenethylamines (2C-X) and their N-(2-methoxybenzyl) (NBOMe) derivatives are becoming increasingly more prevalent in the recreational drug use scene. With the introduction of clandestine websites such as the Silk Road it has made it much easier for users to purchase these substances anonymously. In addition to the easy availability, these rapidly changing analogues make it difficult for laboratories to rely on traditional targeted screening methods for the detection of newer analogues of new psychoactive substances (NPS).
To investigate the collision-induced fragmentation pathways of 2,5-dimethoxyphenethylamines and their NBOMe derivatives using high-resolution mass spectrometry (HRMS) in order to develop a non-targeted strategy for newly emerging compounds of these classes.
In this study, thirteen 2C-X and fourteen NBOMe derivatives including two deuterated compounds (2C-B-d¬6 and 25I-NBOMe-d9) were analysed at different collision energies and their mass spectra evaluated. Chromatographic separation of 1 mg/L standards was achieved using an Agilent 1290 series liquid chromatograph coupled to an Agilent 6510 quadrupole time-of-flight mass spectrometer. A gradient elution was used in combination with an Agilent Poroshell C18 column (2.1 x 75 mm, 2.7 µm particle size). Fragmentation of precursor ions was achieved by positive electrospray ionisation with collision energies set at 10, 20 and 40 eV. An 'auto MS/MS' function was used which applied the collision energy to any observable ions passing through the quadrupole.
For 2C-X derivatives, there is a characteristic loss of ammonia (17.0265 Da) at 10 eV and subsequent losses of CH_3 (15.0235 Da) at 20 and 40 eV. Losses of the para-substituted functional groups (relative to the phenethylamine chain) can be observed in compounds such as 2C-B and 2C-I where it can be seen that a loss of bromine (78.9183 Da) and iodine (126.9045 Da) has occurred after the loss of ammonia. This is also confirmed in the former case where the M+2 peak attributed to the bromine isotope is no longer present. For NBOMe derivatives there are only 2 major product ions formed at m/z 121.0653 and m/z 91.0548 which correspond to the 2-methoxybenzyl cation ($C_8H_9O^+$) and the tropylium ion ($C_7H_7^+$) respectively. The use of deutrerated standards aided in the elucidation of product ion structures.
The information generated in this study may assist laboratories in detecting newer analogues presumptively based on the trends in mass fragmentation patterns without the needs for certified reference materials.

P63. GAS CHROMATOGRAPHY – TRIPLE QUADRUPOLE MASS SPECTROMETRY DETERMINATION OF SILDENAFIL AND ITS ANALOGUES IN DIETARY SUPPLEMENTS

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Development of sensitive and accurate methods to screen and confirm the illegal adulterants in dietary supplements, herbal and food products are of urgent priority. Two approaches maybe used to achieve greater analytical measurement certainty – improved resolution through use of additional separation dimensions, or increased specificity by mass spectrometry. To improve identification power, GC-MS/MS with a triple quadrupole instrument can be used to provide greater sensitivity and selectivity of identification. The use of chemical ionisation increases molecular ion abundance, and with MS/MS achieves adequate identification. A comparative multidimensional gas chromatography experiment achieves quantitative separation of target compounds from matrix constituents.
The aim of this study was to demonstrate a fast identification and quantification method for 6 phosphodiestrase-5 (PDE-5) inhibitors (sildenafil, dimethylsildenafil, homosildenafil, thiosildenafil, thiodimethylsildenafil and thiohomosildenafil) by using GC-QQQMS in dietary supplements. Chemical ionisation MS improved molecular ion abundance for underivatised drugs, which otherwise gave poor MH^+ ions in electron ionisation. The mass spectrometer was operated in multiple reaction monitoring (MRM) mode, for better sensitivity and selectivity. In this manner, the method is adequate to reduce background noise and gave fewer interferences arising from co-eluting compounds in the samples. A multidimensional GC approach was contrasted with the GC-MS/MS method.
GC-QQQMS analyses were conducted by using two different ionisation techniques, electron ionisation (EI) and chemical ionisation (CI). The chromatographic separation was performed on a short 10 m non-polar capillary column, without derivatisation. The dietary supplement sample was prepared by removing the sample from capsules, dissolving it in ethyl acetate, followed by mixing then filtering the residue, before commencing the GC-QQQMS

analysis. Multidimensional gas chromatography was conducted by using a Deans switch system, with heart-cutting of target compounds to a high resolution second column, for interference-free analysis.
Both EI and CI techniques allowed identification and confirmation of each compound; the former gave poor molecular ion abundance. Method validation included limit of detection (LOD), lower limit of quantitation (LLOQ), linearity, precision and recovery. The LOD obtained from EI and CI techniques varied from 0.03 to 1.5 µg/g, with LLOQ ranging from 0.1 to 5 µg/g. Good calibration linearity was obtained for all analogues at a concentration range 0.1 to 20 µg/mL, with correlation coefficients (R^2) higher than 0.99 for both techniques. Mean recovery rates were acceptable for all analytes and the relative standard deviation (RSD) of replicate analyses was less than 15%. The proposed method was applied to the analysis of phosphodiestrase-5 (PDE-5) inhibitors in dietary supplements.
The proposed method showed that by using a triple quadrupole method with MRM mode, the determination of each analogue provided adequate sensitivity, selectivity and acceptable separation without derivatisation. The method was successfully applied to dietary supplement samples. These were found to be adulterated with sildenafil at concentration 3.57 ± 0.37 µg/g in 0.2 g of sample A, and 4.66 ± 1.00 µg/g was detected in adulterated sample B.

P64. CHARACTERIZATION AND IN-VITRO PHASE I METABOLITE IDENTIFICATION OF THE DESIGNER BENZODIAZEPINES CLONAZOLAM, DESCHLOROETIZOLAM, AND MECLONAZEPAM

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Benzodiazepines play an important role in forensic and clinical toxicology as they are widely used for treatment of sleeping and anxiety disorders, seizures and as drugs of abuse. In 2012 the first designer benzodiazepines were offered in Internet shops as an alternative to prescription-only benzodiazepines. Soon after the first of these compounds were scheduled in different countries, new substances were offered, with clonazolam, deschloroetizolam, and meclonazepam being three of the most recent ones.
The present study was set up to characterize three designer benzodiazepines which recently emerged on the 'legal high' market and to investigate their metabolism in-vitro using pooled human liver microsomes (pHLMs). The information gained should also be used to update analytical methods for the detection and identification of benzodiazepines in biological samples.
Deschloroetizolam was obtained as research chemical, whereas clonazolam and meclonazepam were purchased as capsules and tablets, respectively. For identification and characterization of these three benzodiazepines, NMR spectroscopy, GC-MS, LC-MS/MS and LC-Q-ToF-MS were applied. Additionally, IR spectra were recorded. The main phase I metabolites were investigated by incubating 20 µM of each substance for 30 min at 37 °C with pHLMs. Enhanced product ion (EPI) scan experiments, with the hypothetical masses of potential phase I metabolites selected as precursor masses, as well as Q-ToF analysis were conducted.
For all three compounds the declared structural formula was confirmed by NMR spectroscopy. At least one monohydroxylated metabolite could be identified for each compound. A di-hydroxylated metabolite was found for deschloroetizolam. For clonazolam and meclonazepam signals at the m/z corresponding to the reduction of the nitro-group to an amine were observed. Desalkylations, dehalogenations or carboxylations were not observed for any of the investigated compounds. Furthermore, for clonazolam and meclonazepam no metabolites formed by a combination of reduction and mono- or di-hydroxylation could be detected Hydroxylation is most likely to occur at positions known from main metabolites of structurally analogue benzodiazepines with medical use. However, this hypothesis would need to be verified by NMR spectroscopy.
The three benzodiazepines clonazolam, deschloroetizolam, and meclonazepam were structurally characterized and their respective in-vitro main phase I metabolites were identified. Future studies should include verification of the proposed positions of hydroxylation, comparison of the identified metabolites with metabolites formed in-vivo as well as assessment of basic pharmacokinetic data. For other classes of designer drugs like synthetic cannabinoids or designer stimulants it already is inevitable to keep analytical methods up-to-date in order to proof or exclude a consumption or administration of these new drugs. It seems that this now has also become necessary for the analysis of benzodiazepines.

P65. VALIDATION OF A NEWLY DEVELOPED IMMUNOLOGICAL LATERAL FLOW RAPID TEST FOR FAST AND EFFICIENT SOLID SUBSTANCE TESTING (TRACEDETECT)

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Police, customs and prisons frequently need to identify unknown substances (mainly tablets and powders) instantly. Chemical tests already available on the market show both relatively low sensitivity and specificity. Thus, the aim of this study was to validate the new immunological lateral flow solid substance test TraceDetect®, which was especially developed for this purpose.
Both, reference standards and 32 pre-analysed seized samples were used in this study (e.g. Heroin, Cocaine, Crystal, Ecstasy Pills and Amphetamine). Either a trace of the respective substance was taken with a wooden tooth stick or a swab of a contaminated surface was taken and dissolved in the supplied test buffer. The buffer was then transferred onto the lateral flow rapid test. The test was able to detect the five most relevant drug classes: Amphetamines, Cocaine, Methamphetamine, Heroine and MDMA. Results were already readable after 1 minute.

The interpretation of results (positive/negative) was done by visual interpretation of the presence or absence of the respective test line.
The sensitivity of the test was determined to be around 200-300 ng of each drug, respectively. 31 of the samples were identified correctly. It was even possible to discriminate between Amphetamine, Methamphetamine and Ecstasy/MDMA. However, one Cocaine sample showed positive for both Cocaine and Heroin. This sample was reported to have been seized from a dealer who also sold Heroin. In this case, both seizures (Heroin and Cocaine) were stored together and it is most likely that the positive Heroin test came from a contamination of the Cocaine sample with traces of Heroin. No false-positive results were obtained with any cutting agent (e.g. Caffeine, Paracetamol, Lidocaine, Papaverine, Noscapine, and Thebaine). It was not possible to overload the test by applying too much of a substance.
The TraceDetect rapid test can be used for quick and easy screening of the respective substance classes and even further to discriminate between Amphetamine, Methamphetamine and Ecstasy/MDMA.

P66. EFFECT OF METHANOL AS A SOLVENT FOR COCAINE USED AS A REFERENCE MATERIAL

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Cocaine is the most used drug of abuse in Brazil. Therefore, it is object of interest and research in the forensic environment. Due to the great difficulty of acquisition of certified standards of drugs of abuse, it was necessary to purify seized samples in order to use them as reference materials in forensic laboratories of Bahia. These purified samples were used in different analytical procedures like thin-layer chromatography (TLC), gas chromatography-mass spectrometry (GC-MS) and high performance liquid chromatography coupled to diode array detector (HPLC-DAD) employed in the forensic analysis. One of the most recommended organic solvent for solubilization and storage of cocaine pattern is methanol. However, it was observed the appearance of other substances when purified cocaine was stored in this solvent by means of chromatographic techniques.
Studying the reactions that occur with cocaine stored in methanol, in order to identify the substances originated in this chemical enviroment.
Cocaine was purified by TLC, using as mobile phase, methanol:ammonia (100: 1.5, v / v) on silica plates (20cm x 20 cm). The eluate was extracted from the silica with methanol P.A. grade (99.99% with water 0.02%) and hexane BP 99.18%. The extracts were analyzed by GC-MS daily and stored at 7°C in sealed vials.
It was observed that with a day of storage hydrolysis of the cocaine occurred. The identified substances were methylecgonine and methylecgonidine with progression of the areas of the chromatographic peaks over a week, featuring the increase of substances. The same sample was stored under identical conditions, using hexane as the solvent, for a period of three months. It was not observed any cocaine reaction products in this environment. Probably the hydrolysis reaction was promoted by traces of water present in the methanol and the humidity, which reaches 80% on site. The hydrolysis reaction is an equilibrium reaction and is favored by traces of acid, these traces of acid may come following the original sample, purified by preparative TLC. Within 24 hours, was observed the formation of the hydrolysis product (methylecgonine) and dehydration of methylecgonine product, also know as methylecgonidine ester.
Methanol is not a good solvent for storing cocaine due to the promotion of the hydrolysis reaction, producing methylecgonine and causing the dehydration of methylecgonine originating methylecgonidine. Probably methanol easily absorbs the ambient water or holds traces of water capable of providing the reaction. Hydrolysis of cocaine in methanol occur whenever stored at not proper conditions. Thus, the ideal condition is that used in storage a stock solution in a freezer at -20°C.

P67. EVALUATION OF DRUGS OF ABUSE EXTRACTION FROM ORAL FLUID USING SUPPORTED LIQUID EXTRACTION (SLE) PRIOR TO UPLC/MS-MS ANALYSIS

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Drug screening using oral fluid has gained popularity over recent years due to its simple, non-invasive collection means. Screening drugs of abuse can be complicated due to the wide variation of functional groups associated with different analyte classes. Most extraction techniques cannot extract all analytes using a single procedure without using non-optimal extraction protocols resulting in compromised extract cleanliness. Supported liquid extraction allows for the simultaneous analysis of cross functional analytes in a single extraction protocol without forfeiting extract cleanliness.
The aim was to develop a UPLC/MS-MS assay for the determination of a range of drugs of abuse from oral fluid using supported liquid extraction (SLE), after collecting specimens from a variety of collection devices. The devices evaluated were Intercept from Orasure and Quantisal from Immunalysis.
Negative oral fluid samples for method development purposes were obtained using the Immunalysis Quantisal and Orasure Intercept collection devices. To ensure maximum oral fluid extraction on SLE+, samples from Quantisal and Intercept collection devices were modified in pH terms using 10-15µL of concentrated NH_4OH. The target for elevated pH was between 8.2-8.5 to provide a balance for the extraction of the basic drugs but also to avoid any potential hydrolysis of 6-MAM to morphine. Final pH control used 15µL of neat NH_4OH and 10µL 0.5% NH_4OH resulting in loading pHs of 8.3 and 8.5 for the Quantisal and Intercept devices, respectively. Extraction evaluation was performed loading 300µL on ISOLUTE SLE+ 400µL capacity columns, followed by elution with 2x 1 mL of either

MTBE, DCM, 95/5 DCM/IPA or EtOAc. Benzodiazepines, z drugs, amphetamines, cathinones, opiates, cocaine, buprenorphine, THC-COOH, fentanyl and ketamine spiked oral fluid extracts were analysed using a Waters ACQUITY UPLC system coupled to a Quattro PREMIER XE triple quadrupole mass spectrometer. Positive ions were acquired using electrospray ionization operated in multiple reaction monitoring mode.
The DOA multisuite showed optimum extraction at pH condition between 8-8.5. No degradation of 6-MAM was observed in this pH environment. The use of 95/5 DCM/IPA suffered from non-optimal extract cleanliness associated with co-extraction of OF buffer additives and was discontinued for both devices. Optimization of loading volume provided the optimal balance of extract cleanliness and recoveries when using DCM for Quantisal and EtOAc for Intercept. Recoveries were greater than 80% for the vast majority of analytes for both collection devices. BZE recovery was low in EtOAc, however a sub-nanogram LLOQ was achievable in spite of this. Calibration curves were constructed from 1-500ng/mL and good linearity was universally achieved, demonstrating coefficients of determination > 0.99 for both collection devices. When utilizing optimum pH control and extraction solvent for each oral fluid device, it was possible to extract a wide range of drugs of abuse with varying logP and pKa values, demonstrating the possibility of a single extraction.
This poster demonstrates the extraction of a range of drugs of abuse prior to UPLC/MS-MS analysis. The analyte list includes benzodiazepines, z drugs, amphetamines, cathinones, opiates, cocaine, buprenorphine, THC-COOH, fentanyl and ketamine.

P68. EXTRACTION OF PROPOFOL FROM WHOLE BLOOD USING SUPPORTED LIQUID EXTRACTION (SLE) PRIOR TO GC/MS ANALYSIS

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Propofol is used in clinical environments to induce states of anesthesia or reduced sensitivity during surgical procedures. However it also has the potential to be abused for short-term hallucinations and euphoric effects, and with a very small therapeutic range, unanticipated fatal results are possible.
The aim was to develop a GC/MS assay for the determination of propofol from whole blood using supported liquid extraction (SLE). The SLE extraction mechanism is very efficient, delivering higher analyte recoveries and cleaner extracts than equivalent LLE methods.
Blank human whole blood was spiked with propofol and propofol-d17 was used as the internal standard. Extraction conditions were evaluated using spiked whole blood pre-treated 1:1 (v/v) with HPLC grade water. Sample preparation was performed on ISOLUTE SLE+ 400 µL capacity columns using 300 µL of pre-treated whole blood. Extraction was evaluated using heptane, ethyl acetate or MTBE as the solvent of choice. Prior to evaporation under an air stream, 10 µL of 0.5% tetrabutylammonium hydroxide was added to each sample after extraction to evaluate stability during evaporation. Samples were blown down with air below 25°C and derivatization was performed using 100 µL heptane. The samples were vortex mixed and transferred to glass vials with non-split caps prior to GC/MS analysis. All samples were analyzed using an Agilent 7890 GC with a 5975 MSD. Chromatography was performed on a Phenomenex Zebron ZB-Semivolatiles capillary column; 30 m x 0.25 mm ID x 0.25 µm using 1.2 mL/min helium as the carrier. Positive ions were acquired using electron ionization operated in SIM mode.
The presence of tetrabutylammonium hydroxide (TBAH) proved to be essential in comparison evaporation experiments. In the absence of TBAH, it is believed the -OH on propofol becomes ionized during evaporation and analyte losses are almost total. Propofol peak areas from samples that were spiked before and after extraction were compared to determine percentage recovery when using the SLE procedure. Recovery profiles were determined to be greater than 95% and corresponding relative standard deviation (RSD) below 6%. The limit of quantitation was measured to be 2.5 ng/mL on the 400 µL format. In order to increase the sensitivity, the reconstitution solvent volume could be evaluated. Linearity experiments demonstrated coefficients of determination greater than 0.999 over concentration levels 2.5 ng/mL to 1000 ng/mL.
This poster provides a quick, simple and reliable protocol for the extraction of propofol from whole blood prior to GC/MS, demonstrating high, reproducible extraction efficiencies and acceptable limits of quantitation.

P69. EXTRACTION OF DELTA-9-THC, THCA AND 11-NOR-9-CARBOXY-THC FROM ORAL FLUID USING SUPPORTED LIQUID EXTRACTION (SLE) AFTER COLLECTION WITH THE QUANTISAL, INTERCEPT & ORAL-EZE COLLECTION DEVICES PRIOR TO GC/MS ANALYSIS

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Oral fluid testing is gaining approval in the forensic toxicology community as a suitable tool to supplement urine and blood testing where misuse of drugs is suspected. A quick, dignified specimen can be obtained from a person relatively easily in workplace applications, drug driving incidents and other applications. Cannabis misuse continues widely all over the world, and this has led to the necessity for rapid and reliable methods for the analysis and quantitation of THC and its main metabolite 11-nor-9-carboxy-THC (THC-COOH). In addition, THCA is marker which can show a distinction between Cannabis Sativa use and synthetic medicinal Dronabinol.
The aim was to develop a GC/MS assay for the determination of THC, THCA and THC-COOH from oral fluid using supported liquid extraction (SLE), after collecting specimens from a variety of collection devices: Orasure Intercept, Immunalysis Quantisal and Quest Oral-Eze.
THC, THCA and THC-COOH were spiked into oral fluid collection devices following collection of blank oral fluid and

THC-D3 and THC-COOH-D3 were spiked as internal standards. Extraction conditions were evaluated on ISOLUTE SLE+ 400µL capacity columns for all three manufacturers and 1mL capacity columns for Intercept and Quantisal devices, at pH environments native to the device and also modified with ammonium hydroxide. The final optimized procedure involved modifying the pH of the sample before extraction with 10µL 0.5% ammonium hydroxide per Intercept device, 15µL concentrated ammonium hydroxide per Quantisal device and 10µL 4% ammonium hydroxide per Oral-Eze device. Extraction solvents used in development included methyl tert-butyl ether (MTBE), dichloromethane (DCM), 95/5 dichloromethane/isopropanol (DCM/IPA) and ethyl acetate (EtOAc). For all manufacturers, the optimum elution solvent was 95/5 DCM/IPA. For Intercept and Quantisal devices, MTBE was proven to be a suitable solvent where chlorinated solvents are prohibited. Analytes were spiked into blank elution solvent following extraction to determine SLE efficiency. Samples were evaporated under an air stream at 40°C and derivatized using 50µL EtOAc and 25µL BSTFA:TMCS. Samples were analyzed using an Agilent 7890 GC with a 5975 MSD. Chromatography was performed on a Phenomenex Zebron ZB-Semivolatiles capillary column; 30m x 0.25mm ID x 0.25µm using 1.2mL/min helium as the carrier. Positive ions were acquired using electron ionization operated in SIM mode. Recoveries using 95/5 were greater than 76% using Intercept, greater than 78% using the Quantisal and 104-109% using Oral-Eze. RSDs were below 10% across 400µL and 1mL formats using an optimized method. Analyte limits of quantitation using the 1mL format were measured to be 4ng/mL for THC, 10ng/mL for THCA and 20ng/mL for THC-COOH. Linearity experiments over concentration levels 4ng/mL to 800ng/mL, demonstrated R^2 between 0.9920 and 0.9997.
This poster provides a quick, simple and reliable protocol for the extraction of delta-9-THC, THCA and 11-nor-9-carboxy-THC from three oral fluid collection devices prior to GC/MS, demonstrating high, reproducible extraction efficiencies.

P70. **EXTRACTION OF DELTA-9-THC, 11-HYDROXY-DELTA-9-THC AND 11-NOR-9-CARBOXY-THC FROM WHOLE BLOOD AND 11-NOR-9-CARBOXY-THC FROM URINE USING SUPPORTED LIQUID EXTRACTION (SLE) PRIOR TO GC/MS ANALYSIS**

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Cannabis misuse continues widely all over the world, and this has led to the necessity for rapid and reliable methods for the analysis and quantitation of THC and its metabolites in various matrices.
The aims were to develop GC/MS assays for the determination of THC, THC-OH and THC-COOH from whole blood and THC-COOH from urine using supported liquid extraction (SLE).
Blank whole blood was spiked with THC, THC-OH and THC-COOH and THC-D3, THC-OH-D3 and THC-COOH-D3 were used as the internal standards. Extraction conditions were evaluated using spiked whole blood pre-treated 1:1 (v/v) with 0.1% formic acid or 50mM ammonium acetate to give a total of 750µL on ISOLUTE SLE+ 1mL capacity columns. The BeadRuptor24 was evaluated prior to sample application to determine any binding disruption effects. Increased loading volumes and increased percentages of whole blood were evaluated to improve LLOQs. Blank urine was spiked with THC-COOH and THC-COOH-D3 was used as the internal standard. Extraction conditions were evaluated using pre-treatments of 0.1% and 1% formic acid or HPLC-grade water 1:1 (v/v) on ISOLUTE SLE+ 1mL capacity columns. Various urine-hydrolysis approaches were investigated. Beta-glucuronidase enzyme from Helix pomatia (50µL/mL of urine) was used within a pH environment of ~5.5, achieved with ammonium acetate. Also evaluated was 10N potassium hydroxide (KOH) (100µL/mL of urine). Both hydrolysis approaches were performed at temperature; enzyme for 2 hours at 37°C and KOH for 25 minutes at 60°C. Post hydrolysis, the KOH approach required the addition of glacial acetic acid to lower the pH environment to ~5.5. Analyte extraction for both matrices was evaluated using MTBE, DCM, hexane and EtOAc. Both whole blood and urine extracts were evaporated with air at 40°C. Derivatization was performed using 40µL EtOAc and 20µL BSTFA. The samples were vortex mixed and transferred to glass vials with non-split caps prior to heating at 70°C on a heat block for 25 minutes. 1µL was then used for GC/MS analysis in splitless mode. All samples were analyzed using an Agilent 7890 GC with a 5975 MSD. Chromatography was performed on an Agilent J&W DB-5 capillary column; 30m x 0.25mm ID x 0.25µm using 1.2mL/min helium. Positive ions were acquired using electron ionization operated in SIM mode.
Whole blood results using the BeadRuptor24, loading 600µL whole blood and 200µL 0.1% formic acid (aq) for 800µL in total, gave recoveries >74%, with RSDs <9%. LLOQs were 1ng/mL for THC and 3ng/mL for the metabolites. Hydrolyzed and un-hydrolyzed urine extractions gave THC-COOH recoveries >78%, with RSDs <10%. LLOQs were between 6-10ng/mL for unhydrolyzed urine, 10-15ng/mL for KOH hydrolysis and 15-30ng/mL for enzyme hydrolysis. Linearity experiments demonstrated r^2 >0.994 for whole blood and >0.997 for all urine protocols, with concentrations 1-150ng/mL.
This poster describes quick, reliable protocols for the extraction of Δ9-THC, and its metabolites from whole blood and for the carboxy-metabolite from urine prior to GC/MS, demonstrating high, reproducible extraction efficiencies.

Poster abstracts

P71. **SUPERCRITICAL FLUID CHROMATOGRAPHY (SFC) AS ORTHOGONAL TECHNIQUE FOR IMPROVED DETECTION OF POLAR ANALYTES IN ANTI-DOPING CONTROL**

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Since years HPLC-MS/MS gained importance for the detection of various classes of drugs. In contrast to the classical GC-MS technique, it allows for separation of analytes with different functional properties without derivatization. However, some analytes are still challenging as HPLC-MS/MS shows limited resolution capabilities and highly polar analytes interact only insufficiently on the conventional analytical columns. HPLC-MS/MS of some highly polar stimulants proved hard due to very low interaction with the reversed phase (RP) columns generally used for HPLC-MS/MS). Even on the more polar RP phases like C6-phenyl as well as HILIC columns their analysis remained challenging or even impossible. Supercritical fluid chromatography (SFC) as orthogonal separation technique to HPLC may help to overcome these issues.
The aim of the study is to check the applicability of SFC-MS/MS for the analysis of highly polar doping related compounds in urine. The study compares standard reversed phase and HILIC methods with SFC-MS/MS.
A SFC-triple quadrupole system was setup using supercritical CO_2 as mobile phase. Separation of 40 polar compounds (24 beta-adrenergic agents, 12 stimulants and 4 narcotics) relevant in doping control was optimized utilizing different modifiers (methanol, formic acid, ammonia) and different columns. The optimized method was compared to existing reversed phase and HILIC LC-MS/MS methods applied to spiked as well as to post-administration urines. The comparison with reversed phase based methods showed similar performance for most of the drugs analyzed. Retention times and elution orders are different due to the orthogonality of SFC. Very polar compounds such as etilefrine, showing almost no retention under RP conditions, are better retained with the SFC method. This is of advantage especially for direct injection methods with high matrix load. Thereby ion suppression is drastically reduced. Comparing SFC with HILIC resulted in a much better chromatographic performance such as peak shape, retention time stability. Run times on the SFC method been considerably shorter than for the HILIC separation. Retention times and elution orders using the SFC method are different to both reversed phase and HILIC separations due to the orthogonality of the SFC technique. Limits of detection were obtained below the minimum required performance levels in anti-doping control (in general 20 ng/mL for beta-2-sympathomimetics, 100 ng/mL for stimulants and beta-blockers, and 50 ng/mL for narcotics).
With new SFC techniques in place, SFC has a good potential for the analysis of polar compounds. Method comparison to standard reversed phase methods is good and showed much better performance than state of the art HILIC columns and methods. This results in better column lifetime and shorter equilibration time and therefore faster cycle times. As temperature and pressure strongly influence the polarity of supercritical fluids, a precise temperature and backpressure regulation is required for the stability of the retention times. As CO_2 is the main constituent of the mobile phase in SFC solvent consumption and solvent waste are considerably reduced.

P72. **IDENTIFICATION OF 1,3-DIMETHYLAMYLAMINE (1,3-DMAA) IN FOOD SUPPLEMENTS USING DIFFERENT DERIVATIZATION REACTIONS AND ANALYSIS BY GC-MS: A CASE REPORT**

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1,3-DMAA (1,3-dimethylamylamine) is a stimulant used primarily as an aid to weight loss, increased athletic performance and even as a drug of abuse. According to the National Health Surveillance Agency (ANVISA), the dietary supplements marketed in the country containing this substance are not safe for consumption as food and may not be used without medical supervision. The human health problems can include toxic effects (especially liver), metabolic disorders, cardiovascular damage, nervous system disorders and death.
To develop an analytical strategy for the identification of 1,3-DMAA in dietary supplements, using different reactions derivatization and analysis by GC-MS.
Two capsules dietary supplement brand "Jack 3d", seized and sent to forensic analysis, were analyzed. The pulverized content of each capsule was added to two microtubes (A and B) with 1 mL each of methanol, vortexed for 1 min, brought to ultrasound for 15 min and centrifuged for 5 min at 13000 rpm. The supernatant was transferred into two other vials. In The vial was added 500 uL of acetic anhydride and vial B 500 uL of benzaldehyde. The samples were stirred gently until homogenization and stored at room temperature for 20 min. 1 uL of each vial was injected in split mode on an Agilent 5975C GC-MS in full scan acquisition mode m/z 40-550 operating at 70 eV, with a HP-5ms column.
The chromatograms obtained for the two samples containing different products derivatization, showed two well-resolved and symmetrical peaks, but with different retention times (RT). Sample A containing methanol extract of 1,3-DMAA and acetic anhydride showed two chromatographic peaks at 10.19 min and 10.27 min. Sample B containing methanol extract of 1,3-DMAA and benzaldehyde showed two chromatographic peaks at 13.29 min and 13.42 min. Given the presence of two chiral carbons in the molecule of 1,3-DMAA and four enantiomers in the sample, the chromatographic peaks are consistent with the chiral products produced in the reactions, namely (R,R), (S,S) (R,S) and (S,R). Preliminary identification of the derivatives was carried out using the reference and comparison with the mass spectra and subsequent analysis of fragmentation patterns.
The procedure for the derivatization of 1,3-DMAA with acetic anhydride, has proved to be a complementary method to that already established for Thevis et al, using benzaldehyde. The mass spectra analysis of the fragment showed that the products obtained are consistent with the expected products.

P73. **UTILITY AND APPLICATION OF DNA-BASED FORENSIC INVESTIGATIONS IN DOPING CONTROL**

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In doping control it is strictly forbidden the manipulation of biological samples during or after the sample taking. Disputes concerning the assignment of a sample to an individual may arise in the anti-doping context as a consequence of a detected positivity and despite the sampling system is under control by a rigid chain of custody. In this scenario, the implementation of DNA-based forensic identification of samples could represent an helpful tool for the antidoping routine tests. Forensic human identification is based on the analysis of DNA microsatellites or Short Tandem Repeats (STRs). Since they are very variable and 16 loci are tested, the probability that two different individual share the same STR haplotype is virtually null and the identification of a biological sample can be achieved. Moreover, DNA forensic human identification techniques can be applied in other anti-doping context as alternative or complementary methods in the detection of banned substances or doping practices.
We show in this work some applicartion of forensic DNA analysis in specific antidoping fields: 1) for the correlation of athlete's urine sample in longitudinal studies as a complementary, everytime the steroid profile gives ambiguous results. 2) for the identification of samples manipulation. 3) as alternative, sensitive, method for the detection of homologous blood transfusion complementary to the routine method based on flow-cytofluorimetry
DNA is extracted from urine sediment or whole blood using specific extraction kit from Applied Biostystems.STR loci are amplified with PCR using primers labelled with different fluorochromes. STR fragments are separated using an ABI310 capillary electrophoresis system (Applied Biosystems). Steroid profiles were obtained through the analysis in gas-chromatography coupled to mass spectrometry (GC-MS). For homologous blood transfusion detection mixed whole blood samples were obtained simulating a transfusion in vitro at different percentages of the donor. Urine samples used for the comparison of DNA typing and steroid profile were stored at different temperatures and analysed at different times in order to establish the stability of the DNA-based approach compared to the one based on steroid profile.
For the comparison with the steroid profile, results indicate that complete DNA profiles can be achieved also after many days of storage, and the DNA identification is more stable compared to the identification based on steroid profile. Urine samples stored not frozen shows pattern of degradation of the steroid profile in time. DNA degradation instead seems to be a minor problem. For homologous blood transfusion detection, the flow cytofluorimetric method has some limitation for the limited number of blood group analysed so that a perfect haplotype match between the donor and the receiver can occur. DNA analysis allow the recognition of mixed samples in different scalar concentration of the donor and allow to overcome the problems of false negative with absolute certainty
DNA forensic human identification techniques can be considered a powerful tool in a doping control laboratory not only being applied for the identification of biological samples but also as a reliable method of detection for some doping practices.

P74. **GAS PHASE ION SEPARATION OF DRUGS USING A MODIFIER-ASSISTED ELECTROSPRAY IONIZATION-DIFFERENTIAL MOBILITY-MASS SPECTROMETER SYSTEM**

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Differential mobility spectrometry (DMS) is an emerging and rapidly technology for gas phase ion separation. The DMS couple with mass spectrometry (MS) offers great advantages over the use of mass spectrometry alone. Ions are separated based on difference in their mobility between the high field and low field portions of the applied radio frequency voltages. As the ions migrate towards the walls of the DMS cell at different rates, the separation is achieved. When trace quantities of chemical midifiers are added to DMS, their presence modifies the mobilities of analytes of interest through dynamic ion–molecule interactions. This is able to reduce ionization interferences and ionize the target analytes selectively, which provides better mass spectral signal/noise ratios and orthogonal/complementary ion separation to mass spectrometry.
The aim of the study was to validate a differential mobility spectrometry-mass spectrometry system (DMS-MS) system for the simultaneous identification and quantification of amphetamine, methamphetamine, N-ethyl-amphetamine, pseudoephedrine, p-methoxyamphetamine (PMA), p-methoxy-N-methylamphetamine (PMMA), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), and 3,4-methylenedioxy-N-ethyl-amphetamine (MDEA) in urine. The quantity of target analytes in the unknown set was determined using gas chromatography-mass spectrometry (GC-MS) as the reference methodology.
Blank, unknown samples and controls were prepared from 0.5 mL of urine. Twenty µL of 10 µg/mL amphetamine-d8 and MDA-d5 as internal standards (IS) were spiked to all specimens. The samples were infused and ionized using electrospray followed by DMS-MS for gas phase separation. The optimization of ion/molecule chemistry in the DMS was investigated using different alcohols as chemical modifiers. The analysis was performed on a triple quadrupole linear ion trap using the multiple reaction monitoring mode for quantification as well as for confirmatory analyses. The criteria followed here were recommended by SOFT/AAFS Forensic Laboratory Guidelines, 2006.
The optimization of DMS-MS was shown to result in improving peak capacity, separation, and sensitivity. A higher proton affinity modifier (hexanol) provided better peak capacity and separation. A modifier with low proton affinity (methanol) suppressed the analyte ion/modifier proton transfer, which increased signal intensity and sensitivity significantly. The limits of quantitation (LOQ) ranged from 1.0 ng/mL to 5.0 ng/mL, and 0.5 ng/mL to 1.0 ng/mL for the limits of detection (LODs). The linearity was investigated in the range from 1 to 5000 ng/mL for each compound.

The calibration curves exhibited coefficients of determination (R^2) ranging from 0.9984 to 0.9999. Relative standard deviations (RSDs) were less than 15% for intraday (n=5) and interday (n=15) measurements. Ten real samples, which were collected from drug abusers, were confirmed the administration of amphetamine, methamphetamine, MDA and MDMA using GC-MS. Compared with GC-MS, the results were within ±35% difference of the concentrations obtained from GC-MS.
A rapid and sensitive DMS-MS technique has been developed successfully for the identification and quantification of 9 drugs of abuse in urine using chemical modifiers. The method takes less than 5 min for analysis. It requires neither chromatographic separation nor time-consuming sample pretreatment. DMS-MS has emerged as an attractive alternative to conventional 2D analytical techniques, such as liquid chromatography-mass spectrometry (LC-MS) with much shorter analysis times.

P75. **DEVELOPMENT AND VALIDATION OF TARGETED DRUG SCREENING METHOD FOR BLOOD SAMPLES BY LIQUID CHROMATOGRAPHY-TIME OF FLIGHT/ MASS SPECTROMETRY**

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A simple and sensitive method for routine screening of drugs and metabolites is necessary for forensic toxicology laboratory. Therefore, the targeted screening method by liquid chromatography-time of flight mass spectrometry (LC-TOF/MS) was developed for screening 157 drugs in blood samples. These compounds consist of benzodiazepines, antidepressants, amphetamines, opiates and some other class of drugs, which are commonly encountered in forensic cases.
The aim of this study was to develop and validate LC-TOF/MS based screening method for detection of drugs in blood samples.
Blood samples (0.5 ml) and trimipramine (200 ng/ml) as ISTD were extracted with butyl chloride for acidic and basic drugs then pool extractions were analyzed by LC-TOF/MS. Chromatographic separation was achieved on Phenomenex C18 column (2.1x150 mm, 5 um) using Bruker MicrOTOF2 mass spectrometer ESI positive mode. A mass error within +/- 5 mDa, retention time within +/- 0.2 min with an isotopic pattern fit that generate score +++ from target screening software was considered as a tentative positive result. In addition, the fragmentation spectra generated from in-source collision-induced-dissociation (ISCID) at capillary exit voltage of 90V and 160V were also compared with standards for additional confirmatory purposes.
The method was successful for separation and detection of targeted 157 drugs and metabolites including benzodiazepines, antidepressants, amphetamines, opiates, and many other forensically significant substances. Compounds such as THC carboxylic acid, barbiturates and GHB were not identified by this method due to the analysis in positive ionization alone. When ISCID patterns were applied for further confirmation of findings, compounds such as amphetamines, antidepressants can be achieved for this purpose. While compounds like benzodiazepines and opiates did not give any useful fragmented ions. However, this parameter is limited used; because of impurities of fragmented patterns in some samples. In this study, trimipramine was used as ISTD because it was not found in our previous cases. However, trimipramine-d3 might be of interest in future work. Validation was performed on spiked blood samples for selectivity, carry-over, limit of detection (LOD) and matrix effect. Selectivity of the method was estimated by analysis of 10 different blank matrixes and no significant interferences were seen in blank samples. Carry-over was evaluated by analysis of blank samples after spiked sample at concentration of 1000 ng/ml of each drugs and no carry-over were observed. LOD ranged from 5-50 ng/mL for all analytes. Matrix effects in general were found to be acceptable (+/- 20%), except in some drugs, ion suppressions were found. Nonetheless LODs were considered adequate for forensic toxicology purposes. This method was applied to a number of actual forensic samples confirming that the results produced from this method were consistent with results obtained from routine LC-MS/MS method.
The presented LC-TOF/MS method showed to be appropriate for multi-targeted screening of drugs important in forensic cases. With the ability for both targeted and non-targeted screening, LC-TOF/MS can be an ideal tool for forensic toxicology screening.

P76. **STUDIES OF THE VOLATILITY OF AMPHETAMINE AND ITS DERIVATIVES METHAMPHETAMINE, MDA, MDMA, AND MDEA BY VACUUM CENTRIFUGATION AND GC-MS ANALYSIS**

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Most work-up procedures in bioanalytical chemistry are based on an enrichment of analytes due to precipitation, liquid-liquid or solid-phase extraction. Usually, the analytes are therefore transferred in organic layers and evaporated to dryness under a stream of nitrogen or vacuum conditions and increased temperature. For some analytes, especially volatile or unstable compounds, these procedures may be risk the loss of analytes during the sample work-up procedure. The problematical volatile behaviour of the amphetamines are often entitle as well-known, but no comprehensive studies have been published so far.
The aim of the presented study was the investigation of the volatility of amphetamine and its derivatives methamphetamine, MDA, MDMA, and MDEA under common sample work-up conditions in bioanalytical chemistry.
A heated vacuum centrifuge equipped with a temperature and vacuum control system and a cold trap was used to investigate different temperature (35 and 50 °C) and time (23-68 min) conditions for evaporation the solvents with or without matrix (urine and blood, SPE and LLE). Prior to analysis, the samples were spiked with 500 ng of

amphetamine, methamphetamine, MDA, MDMA, MDEA, and 50 ng of its deuterated (d5) analogues, respectively. After microwave assisted HFB-derivatization, the studied compounds were analyzed by EI-GC-MS. Quantitative evaluations were performed using the absolute peak areas of the corresponding analytes.
For all ten compounds, no significant decrease in absolute peak area of analytes in solvents samples without matrix could be observed up to an absolute drying time of 68 minutes under 6 mbar and 50°C (extreme conditions). In contrast to the "solvent samples", matrix containing samples showed under identical conditions higher standard deviation and lower peak areas with increased drying time. Despite these losses under extreme conditions, a robust drying-procedure under moderate conditions (40°C, 30 min for drying 2.0 ml of dichloromethane) could be developed and applied to routine forensic analyses of amphetamines. Possible explanations for the differences in volatility of solvent samples vs matrix containing samples will be presented and discussed in detail.
The study showed the matrix dependent decrease of peak area for all investigated compounds and gives new insights in the evaluation of the volatility of amphetamine derivatives. Evaporation procedures in bioanalytical methods should therefore more investigated during the validation process.

P77. DEVELOPMENT OF A MULTI-PARAMETER ANALYTICAL APPROACH FOR THE DETECTION OF "INDUCED HYPOXIA" IN BLOOD DOPING

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The effect of induced hypoxia, as a consequence of external stimuli, may be used to enhance athletic performance. For this reason the WADA introduced Hypoxia-inducible factor (HIF) stabilizers and activators in the list of prohibited substances and methods. For indeed, Section 2 at point 2 specifically mentions that HIF stabilizers, e.g. cobalt and FG-4592; and HIF activators, e.g. argon, xenon" are prohibited in sport doping". The effect of stabilizers and HIF activators is analogous to that of a blood doping practice. These drugs and some other chemical compounds (such as cobalt) have a quick action and are rapidly eliminated from the body; however their effect is long lasting. HIFs are crucial elements for erythropoietin (EPO) regulation and an attractive site of action for blood doping-like practices.
The goal of this study is to analyse innovative biomarkers that allow to identify a solid and reproducible signature of blood doping, stimulated by treatments of induced hypoxia. The analysis approach we propose is aimed to the identification of robust and long lasting biomarkers, detectable in body fluid, to identify traces of hypoxia treatment even far from the last application. This study is founded on mRNA and microRNA detection principally in blood matrix but with the idea to extend the analysis also to urine, after the signature of hypoxia has been successfully identified in blood.
An in vitro model with human hepatocellular carcinoma cell line (HepG2) has been developed, and first pilot experiments have been attempted treating cells with deferoxamine at concentration of 500 µM and at six different times (2h up to 48h), replied with different time-dependent gene expressions.
From the data we collected, carbonic anhydrase IX (CA IX) has emerged as well expressed after hypoxic condition, while other factor did not show a constant variation at all time of analysis. Results we achieved indicate differential gene expression for all factors analysed. Correlations between protein values and haematological analysis have shown a positive correlation between prolyl hydroxylase 2, hemoglobin and hematocrit; and on the contrary of protein/protein correlation, HIF1 alpha and HIF2 alpha seems to have a different behaviours regarding hemoglobin and erythrocytes, while they agree regarding on %reticulocytes.
All the results obtained from the whole expression patterns of the molecules considered, taking into account all the information given by gene expression transcription (represented by mRNA level), translation (represented by protein level), and regulation (represented by miRNA expression and production) may constitute the basis for a general investigation method to detect "induced-hypoxia" blood doping.

P78. RECENT ADVANCES ON THE APPLICATION OF GC-IRMS IN ANTI-DOPING ANALYSIS: STABILITY AND UTILITY OF LONGITUDINAL DATA

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"Pseudo-endogenous" steroids are synthetically produced steroids that are naturally present in the organism, primarily among them testosterone and its precursors/metabolites. The confirmation of the abuse of pseudoendogenous steroids in anti-doping analysis is based on the analysis by GC-C-IRMS. The current WADA criteria to report an AAF are based on the population distribution of the selected target compounds (TC) compared to the values of the endogenous reference compounds (ERC) (delta-delta ERC-TC < 3‰). The overall methodology is based on the different content of 13C of the synthetic steroids (-33 to -27 ‰) compared to the endogenously produced (-26 to -19 ‰). However there is an intermediate zone (-26 to -23) were the application of the current limits lack of efficacy due to i) the distance between the endogenous steroids and the synthetic is below 3 ‰; ii) non pharmaceutical preparations of synthetic steroids fall in this intermediate range.
In the present work we studied the stability of the IRMS parameters of healthy individuals in terms of short, intermediate and long terms variability over 1 year, with the aim to assess whether they can considered in the framework of the endocrinological module of the Athlete Biological Passport (ABP) in doping control analysis.
A reference population of non-athletes has been monitored with specific reference to the short, medium and long term stability of the 13C delta- and delta-delta-values. The data obtained were compared with those obtained

on samples of a population of athletes undergoing longitudinal tests for atypical steroid profile but eventually confirmed as negative. Data were obtained on as many as seven target compounds and four endogenous reference compounds.
Our results show that the variability obtained for the IRMS data is lower than the one observed for the parameters of the urinary steroid profile presently included in the ABP. In addition for a given individual, the maximum acceptable variation of the IRMS data is much lower than the population reference limits. This should permit to reduce the gap between the suspicion of an atypical profile and the confirmation capacity by IRMS, and eventually detect the abuse of formulations of synthetic steroids showing delta values close to the endogenous region.
Our result indicate that evaluation of the IRMS data in addition to those of the urinary concentration of the markers used to define the urinary steroid profile, and, consequently, its inclusion on the endocrinological module of the ABP, may be of help in order to extend the detection window of pseudo-endogenous steroids.

P79. LSD SAMPLE CHARACTERIZATION WITH SUBSTANCES SUSPECTED COUNTERFEIT TYPE NBOME

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LSD is a recreational drug which is commercialized in pieces of blotting paper. Currently, the people who use this kind of substance have reported effects different to those which have usually been recognized for LSD samples. In 2013, Colombian Ministry of Justice through the Warning System for emerging drugs, has reported the detection of 25C-NBOMe and 25B-NBOMe substances in samples of LSD type in Bogota and the Colombian coffee growing axis. The NBOMe substances are phenethylamines derived from hallucinogens of 2C type (4-X-2, 5-dimethoxyphenethylamine), powerful partial agonists of the 5-HT2A receptors of serotonin.
To document the detection and characterization of NBOMe substances in samples of LSD drugs commercialized in Bogota during the first half of 2014, through the use of gases' chromatography coupled to spectrometry mass.
Fragments of blotting papers with printed illustrations were the materials analyzed. A blotting paper was extracted in 1 mL of methanol in ultrasound for 15 minutes. The supernatant was analyzed by adding tetracosane like internal standard. The GC–MS analysis was made by using a GC 7890A chromatograph of gases coupled to a selective detector of mass, 5975C series, which is manufactured by Agilent Technologies. The extracts were injected automatically in Split mode. Chromatographic separation was carried out on a HP-5MS capillary column (30m x 0.25mm x 0,25µm) by using helium at a constant rate of 1 ml/min as the carrier gas. The initial column temperature (100°C) was maintained for 2.5 min, and then it was increased linearly at a rate of 25°C/min to 230°C staying for 1 min. Finally, it was increased linearly at a rate of 35°C/min to 290°C staying for 9 min. The spectrometer was operated in electron impact mode (EI). The temperatures of ion source and the quadrupole were 200°C and 280°C, respectively. Ionization energy was established at 70 eV and the positive ions were analyzed. The acquisition was carried out in scan mode from 35 to 450amu.
Taking into account the chromatographic conditions employed in the research, it was possible to detect DOC, 25C-NBOMe, 25B-NBOMe, 25I-NBOMe. The retention time for 25C-NBOMe was 11.7 min; for 25B-NBOMe was 11.7 min; for 25I-NBOMe was 11.7 min and for DOC was was 8.2 min, 25C-NBOMe was 12.0 min, 25B-NBOMe was 12.5 min, 25I-NBOMe was 13.2 min, and LSD was 17.2 min.
Through analysis by mass spectrometry GC-EI-MS, it was identified the presence of 25C-NBOMe, 25B-NBOMe, 25I-NBOMe and DOC in blotting papers samples collected in Bogotá which were commercialized as LSD drug in the first half of 2014.

P80. CAMOUFLAGED COCAINE FOR INTERNATIONAL TRAFFIC

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Extraction, detection and quantification of cocaine, hidden in different types of matrices (like solids, liquids, emulsions, suspensions, rubbers, etc.) are critical issues due to the impact on the legal field. Within Narcotics Group of INMLCF (Colombia) are validated and accredited techniques that enable the identification and quantification of cocaine embedded in different materials. Retrospective result analysis for different cases of seizures can take a part of different modalities used for illegal drugs trade and the type of adulterants used to "cut" this drug could be used by consumers outside Colombia.
The aim of this paper is to present the different types of camouflaged cocaine, used to transport cocaine out of Colombia, as well as identify the principal adulterants in this samples.
In order to identify substances, visual inspection of each of the evidence was performed, preliminary tests (cobalt thiocyanate, dragendorff and marquis) were made and then a confirmatory technique (GC-MS) was used.Each sample was fragmented and extracted with ethanol in ultrasound by 15 min, the supernatant was analyzed by GC-MS, tetracosane was added as internal standard. Gradient elution was performed by HP-5MS capillary column in split injection mode, the ionization of the sample was performed by electron impact at 70 eV in scan mode (35 to 450 a.m.u.). The GC-MS technique is validated and accredited. To perform this analysis, 62 cases were randomly selected from the years 2012-2014.
After analyzing the samples, the main results showed that different modalities for cocaine camouflaged used in the test sample are: Solid 17.7%, textile 16.1%, paper 14.5%, fingerstalls (when the human body is the transportation) 14.5%, polymer 11.3%, liquid 11.3%, semisolid 6.4%, rubbers 6.4%, gel 1.6%. Among the 62 samples, one of them contained non-detectable narcotic, another one had lidocaine without controlled substance and only a sample

corresponded to heroin mixed with caffeine. Finally, the main adulterants detected were: Levamisole 38.7%, caffeine 4.8% and lidocaine 9.7%. 51.6% of the samples correspond to cocaine without any adulterant. In the case of fingerstalls 55.6% of the samples contained cocaine with levamisole.
To conclude, there are a variety of ingenious methods used for smuggling cocaine, in order to avoid international controls, among which are the physical camouflage, solutions, heterodisperse and impregnated systems. The main cocaine adulterant in illicit traffic is levamisole; however, the percentage of cocaine without any adulterant is greater.

P81. DETECTION AND IDENTIFICATION OF NEW PSYCHOACTIVE SUBSTANCES IN POOLED URINE USING LIQUID CHROMATOGRAPHY COUPLED TO HIGH RESOLUTION MASS SPECTROMETRY

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Liquid chromatography (LC) coupled to a quadrupole time-of-flight mass spectrometry (QTOF-MS) under data-independent acquisition mode (All-ions MS/MS) provides sensitive, full-spectrum MS and MS/MS data with high mass accuracy allowing the possibility of detecting a large number of compounds in one injection. New Psychoactive Substances (NPS) are compounds that mimic effects of illicit drugs and are synthesized to evade law enforcement by introducing slight modifications to chemical structures of controlled substances. Currently, there are around 450 NPS in the market, their use is very sporadic depending on popularity amongst users. Their detection is a challenge due to their transience on the drug scene, creating a continually moving analytical target. Collecting anonymised pooled urine samples from stand-alone street urinals placed in nightlife locations and screening them for NPS has been proposed as an alternative technique to detect consumption of NPS.
The aim of this work was to show the role of pooled urine in determining commonly used NPS in different locations. In addition, we demonstrate the utility of a suspect screening approach in the detection and identification of different families of NPS and their metabolites.
The LC system consisted of an Agilent 1290 Infinity LC, coupled to an Agilent 6530 Accurate-Mass QTOF MS. The LC separation was performed on a Phenomenex Biphenyl column (100 mm x 2.1 mm, 2.6 µm). The acquisition was performed in positive and negative ionization mode with 3 scan segments at 0, 15, and 35 eV with fragmentor at 100 V at a rate of 2.5 spectra/s. An algorithm based on a suspect screening post-acquisition data processing strategy was developed and linked to an in-house developed database with >1500 entries containing > 350 NPS, their metabolites, and common product ions. This allowed the tentative identification of compounds detected without the need for acquiring reference standards (which are sometimes not available). A total of 20 pooled urine samples collected from various locations in London, UK were used for the application of the method. Urine samples were prepared by overnight hydrolysis with β-glucuronidase at 37 °C followed by a protein precipitation step with acetonitrile (1:2) and evaporated to dryness under gentle nitrogen stream at 40 °C and finally reconstituted in 60 µL of water: acetonitrile (98:2, v/v).
With this suspect screening approach, more than 30 NPS and metabolites from several groups were detected, including phencyclidine-type substances, phenethylamines, piperazines, synthetic cannabinoids, synthetic cathinones, pyrrolidinophenones, and tryptamines. These compounds were tentatively confirmed by the elucidation of accurate MS/MS fragments and ten NPS were eventually confirmed after injection of reference standards. Therefore, this approach has proven ideal for screening purposes.
We demonstrate the capability of suspect screening approach in detecting NPS and their metabolites. Additionally, we show that pooled urine analysis can be a useful technique to determine circulating NPS in a specific region, although only applicable to male subjects.

P82. LIQUID CHROMATOGRAPHY: HIGH RESOLUTION MASS SPECTROMETRY ANALYSIS OF TOXICOLOGICALLY SIGNIFICANT COMPOUNDS IN URINE AND SERUM

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Large number of possible analytes complicates fast and precise identification of unknown compounds in biological samples – main task of forensic and toxicological laboratories. Current use of "new drugs", analogues of forbidden substances, extends amount of compounds probably present in any sample. An analysis using liquid chromatography coupled with high resolution mass spectrometry significantly helps to solve problems with identification.
The high resolution mass spectrometry together with libraries of MS and MS/MS data enables identification of analytes without standards. However, in some cases the comparison with standard is advantageous. In our work the standards of some drugs have been analysed to complete the data in the library and new liquid chromatography – high resolution mass spectrometry method for identification of more than 200 toxicologically significant substances has been developed.
The chromatographic separation of more than 200 representatives of antidepressants, neuroleptics and other drugs, as well as drugs of abuse, has been achieved using Agilent 1290 Infinity UHPLC system and a C18 column (Zorbax Eclipse Plus C18 RRHD (2.1mm x 100mm; 1.8µm), Agilent). 5mM ammonium formate with 0.1% formic acid (A) and methanol containing 0.1% formic acid (B), have been chosen as mobile phases. The following gradient has been employed (all steps linear): 0 min - 95:5 (A:B); 0.5 min - 95:5; 1.5 min - 70:30; 11 min - 0:100, followed by 1.5 min of 100% solvent B and then 2.5 min of the starting conditions. The flow rate has been 0.3 mL/min and the injec-

tion volume 2 µL. A Q-TOF mass spectrometry (Agilent 6550 QTOF) with positive ion electrospray ionisation has been used for identification of the analytes. The method has been designed for analysis of psychoactive substances in rat and human serum and urine respectively, with simple and fast sample treatment used. Urine samples have been analysed after dilution with 0.1% formic acid in a ratio 1:9 and enzyme hydrolysis, respectively. Samples of serum have been treated by protein precipitation with acetonitrile followed by centrifugation. Validation of the method was conducted with samples of human urine and serum of volunteers who did not take any of studied compounds. The limits of quantification (LOQ) have ranged from 0.1 ng/ml to 10 ng/ml. The responses of all analytes have been linear in the range from LOQ to 100 ng/ml of urine or serum. Measured spectra have been compared with those available in libraries for confirmation of the presence. Missing product spectra have been added to the library for almost thirty analytes, as well as retention times for all compounds, which have enable more precise identification, mainly for analytes where the comparison of mass-spectrometry data is not sufficient. Three new drugs have been completely added to the library.
A liquid chromatography – high resolution mass spectrometry method has been developed for analysis of biological samples for precise identification of more than 200 compounds. The method is suitable for identification of drugs in human urine and serum.

P83. EVALUATION OF PAPER SPRAY IONIZATION SOURCE FOR SCREENING OF DRUGS OF ABUSE IN BLOOD AND URINE COUPLED TO HRAM MASS SPECTROMETER

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Forensic toxicologists are always looking for quicker and easier analytical tools to generate fast and accurate results. Immunoassays are fairly quick and easy, but lack selectivity within drug classes. Mass spectrometry offers greater selectivity, but can be more cumbersome and time consuming both in sample preparation and analysis time. Paper spray is a direct ionization source that can give results within one minute per sample and does not require sample preparation. Since it is a direct ionization source, coupling it to a high-resolution tandem mass spectrometer (HR-MS/MS) improves method selectivity.
To evaluate a paper spray ionization source coupled to an Orbitrap-based HR-MS/MS for rapid analysis of drugs of abuse, and to compare the results to those obtained with a conventional HPLC-HR-MS/MS method.
BBlood and urine samples were fortified with internal standard and directly spotted onto a Velox Sample Cartridge (VSC) and dried at ambient temperature for at least 30 minutes. Cartridges were loaded onto a Velox 360™ paper spray source (Prosolia, Inc, Indianapolis, IN) for sample introduction into a Thermo Scientific™ Q Exactive™ Focus hybrid quadrupole-Orbitrap mass spectrometer. The Q Exactive was operated in full-scan data-dependent MS2 mode. In this mode, high-resolution, full-scan data at resolution of 70k were collected and then MS2 spectra at a resolution of 17.5k were triggered for compounds entered in the inclusion list. Data were acquired with Thermo Scientific TraceFinder™ software, version 3.2 and analyzed with Thermo Scientific ToxFinder™ software, version 1.0. ToxFinder software identified compounds based on exact mass of precursor, isotopic pattern and MS2 spectra. Semi-quantitation can be performed either by using a single point calibrator or by using internal standard ratio.
Limits of detection were comparable to but slightly higher than those obtained with an HPLC-HR-MS/MS method on the same instrument. However, most were still within cut-off requirements. Some compounds did not perform well under standard conditions and required additional method development or specifically optimized methods.
Paper spray allows for rapid analysis of many compounds and gives relevant sensitivities. Paper spray shows utility as a fast screening tool for forensic laboratories.

P84. AN LC-MS/MS LIBRARY FOR THE DETECTION OF NEW PSYCHOACTIVE SUBSTANCES

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In recent years, new psychoactive substances (NPS) have been introduced at an increasing rate. In 2014 alone, 101 new compounds have been notified to the EMCDDA for the first time. Expansion and subsequent revalidation of targeted methods for NPS detection thus present a significant challenge in terms of time and financial costs.
Our goal was to develop an LC-MS/MS library for the detection of NPS to be used in conjunction with untargeted methods using information-dependent acquisition (IDA). One aim was to eliminate the need for laborious method revalidation upon method expansion that is commonly encountered with targeted (MRM) methods. In contrast to quantitative LC-MS/MS using MRM methods, for screening-methods based on IDA and library searching less exhaustive method validation is necessary when adding new upcoming compounds to a mass spectral library, since the data acquisition method does not change.
Spectra of pure reference compounds were recorded either following a 5 min chromatographic run on a Synergi Polar-RP column (50 × 2.0 mm, 4 µm) (Phenomenex) or after flow injection. Mass spectral data was acquired using a QTrap 3200 instrument (Sciex) with positive electrospray ionisation. Enhanced product ion (EPI) spectra were recorded with collision energies 20, 35 and 50 eV as well as with collision energy spread 35 ± 15 eV. For HU-210 spectra were also recorded with negative ionisation. For bromine-containing compounds, spectra were additionally recorded for the 81Br precursor. Background subtraction was performed for all spectra before inclusion in the library.
The library in its current version contains data for 387 different compounds, including phenethylamines, ampheta-

mines, cathinones, piperazines, synthetic cannabinoid receptor agonists, tryptamines, opioids, dissociatives, and others including also metabolites. Expansion of the library is an ongoing effort.
We successfully developed an LC-MS/MS library covering a broad range of NPS and their metabolites, including compounds that appeared only very recently. PDF versions of the spectra for manual comparison and reference are freely available online at www.legal-highs.ch. A database file for use with the Sciex Analyst software is available from the authors for forensic and clinical labs upon request and free of charge. Acknowledgements: This research was sponsored by the Swiss Federal Office of Public Health (BAG project 12.009410) and the Drug Prevention and Information Programme of the European Union (JUST/2011/DPIP/AG/3597).

P85. ANALYSIS OF 15 SYNTHETIC CANNABINOIDS AND THEIR METABOLITES IN WHOLE BLOOD BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY AFTER LIQUID-LIQUID EXTRACTION

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Synthetic cannabinoids, sprayed on herbal mixtures, have been abused as new designer drugs in Turkey since 2011. The analysis of synthetic cannabinoids in whole blood is of particular importance in the fields of forensic and clinical toxicology.
In order to investigate the presence of synthetic cannabinoids in blood samples, a liquid chromatography-tandem mass spectrometry (LC-MS-MS) method was developed using 200 µL of whole blood sample. In this study, a determination method was developed and validated for qualifying JWH-018, JWH-073, JWH-081, JWH-019, JWH-122, JWH-200, JWH-203, JWH-210, JWH-250, JWH-398, AM-2201,RCS-4, RCS-8, UR-144, XLR-1 and their metabolites in blood samples.
After addition of 10 µl IStd solution to 200 µL synthetic cannabinoid spiked blood samples, extractions were performed by adding 100 µL buffer solutions with four different pH (0.1 M phosphate buffer of pH 4, pH 7, pH 10, pH 12) and ethyl acetate, ethyl acetate:hexane (80:20, v/v), ethyl acetate: acetonitril (75:25, v/v) and tert-butylmethyl-ether extraction solvents separately. Specimens were reconstituted by 200 µl mixture of mobile phases consisting of 10% mobile phase A (0.01% formic acid in water) and %90 mobile phase B (0.01% formic acid in ammonium formate-acetonitrile). Shimadzu Prominence HPLC system and ABSciex (®) 4000 Qtrap Mass Spectrometer LC-MS/MS instrument with an electrospray source was used for analysis and 20 µl injections were performed to acquire data in positive ionization mode. Gradient chromatographic seperation was achieved utilizing Restek Allure PFPP column with 0.5 ml/min flow rate and overall run time of 17.5 min.
Four different pH buffer solutions and four different extraction solvents were used in the method and all extractions were compared and assessed by recoveries of 15 synthetic cannabinoids with their metabolites in whole blood samples. The highest recovery was evaluated for ethyl acetate:acetonitril (75:25, v/v) solvent extraction.
This extraction method is beneficial to analyze small quantities of blood samples and also very useful and applicable for simple and faster analysis of sythethic cannabinoids in forensic and clinical toxicology laboratories.

P86. EVALUATION OF CAPILLARY DRIED BLOOD SPOT SAMPLING FOR QUANTIFICATION OF COMMON ANTIPSYCHOTICS

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Dried blood spot (DBS) sampling has been proposed as an alternative for classical blood collection for quantification of antipsychotics. A DBS method for determination of 15 antipsychotics and 7 metabolites was developed and analytically validated according to FDA and EMA guidelines (Patteet et al. DTA 2014). However, a comparison between serum, venous whole blood and capillary blood obtained from psychiatric patients was lacking.
A clinical validation of the previously developed DBS method was mandatory before implementation in routine practice.
Serum, venous whole blood and fingerprick capillary blood was collected from patients with the diagnosis of schizophrenia, schizo-affective or bipolar disorder at 3 psychiatric hospitals in Belgium. Patients had to be in 'steady-state' condition, which means that the dose of their antipsychotic drug may not be changed in the last 7 days before blood withdrawal. All samples were taken at the same time just prior to the morning dose of the antipsychotic (trough concentration). Serum and whole blood samples were analyzed using our conventional UHPLC-MS/MS method (Patteet et al. CCA 2014). First, blood:serum ratios were calculated, compared with literature and whole blood therapeutic reference ranges were defined. Second, venous whole blood concentrations were compared with venous blood spotted on DBS (v-DBS) and capillary blood spotted on DBS (c-DBS) by calculating ratios and by Passing-Bablok regression analysis. Finally, the obtained blood levels were evaluated by clinical interpretation of the results using the therapeutic reference ranges.
111 patients (75 male, 36 female; age 19-65 years) were included in the study. From 100 patients, serum, whole blood and v-DBS were collected. From 73 of these 100 patients, also c-DBS was taken. Eleven antipsychotics were found: amisulpride, aripiprazole, bromperidol, clozapine, haloperidol, olanzapine, paliperidone, pipamperone, quetiapine, risperidone and zuclopenthixol. Except for olanzapine, the calculated blood:serum ratio was in accordance with literature. Whole blood therapeutic ranges can be calculated from serum therapeutic ranges (defined in literature) using this ratio. It was demonstrated that concentrations obtained by DBS analysis were highly comparable to those obtained by conventional whole blood analysis by calculating DBS:blood ratios and Passing Bablok

regression analysis. Clinical interpretation of DBS concentrations using therapeutic ranges will result in a reliable conclusion, which will be almost identical to the interpretation of venous and serum concentrations (sensitivity of 92.2% for venous whole blood vs. v-DBS, 95.8% for venous vs. c-DBS, 97.6% for v-DBS vs. c-DBS, and 91.6% for serum vs. c-DBS).
The use of capillary DBS sampling for determination of commonly prescribed antipsychotics was evaluated for the first time. This study demonstrated that DBS sampling is a promising alternative for classical blood sampling and can be used in routine practice for both forensic and therapeutic drug monitoring purposes.

P87. THE DEVELOPMENT OF A MOLECULARLY IMPRINTED POLYMER FOR SELECTIVE EXTRACTION OF SYNTHETIC CATHINONES FROM URINE BY SOLID-PHASE EXTRACTION

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The rapid clandestine synthesis, appearance on the illegal market, and subsequent use of new synthetic cathinones is accompanied by unknown physiological effects and the potential for serious health consequences. The selective extraction and detection of synthetic cathinones in samples of forensic and toxicological interest is critical. Molecular imprinting technology uses macroporous polymers containing binding sites highly selective toward target analytes, and has been reported for use in solid-phase extraction of biological samples.
The aim of the present study was the preparation of a novel molecularly imprinted polymer (MIP) using methacrylic acid as functional monomer and 4-methylmethcathinone as the original template molecule during development. The application of the sorbent material to solid phase extraction (SPE) cartridges was validated, and allowed for selective extraction of the synthetic cathinone in urine prior to High Performance Liquid Chromatography (HPLC) analysis.
The MIP was prepared using methacrylic acid (MAA) as functional monomer, ethylene glycol dimethacrylate (EGDMA) as cross-linker, N,N-azobisisobutyronitrile (AIBN) as initiator and 4-methylmethcathinone (4-MMC) as template molecule by thermal and UV polymerisation methods. The optimal conditions required were determined by performing a series of rebinding studies on: molar ratio between the template, monomer and crosslinker; type of polymerisation method; incubation solvent type; and amount of polymeric material used. MISPE protocols were proposed following further optimisation studies to determine the most effective loading, washing and eluting solvents. Finally, the proposed MISPE protocol was applied to the analysis of spiked human urine.
Binding studies were used to evaluate the binding affinity of the MIP toward the template molecule through a comparison with a non-imprinted polymer prepared under identical conditions, without the addition of the template molecule during pre-polymerisation. A Scatchard analysis showed the imprinted polymer had greater binding associations. Selectivity studies showed the binding of structural analogues of the template molecule were less significant, while non-structural analogues showed poor binding ability. The MISPE effectively extracted the target molecule from a complex matrix, resulting in a much cleaner chromatogram, free from interfering compounds. A validated MISPE protocol was proposed for clinical and forensic toxicology purposes.
A novel MISPE sorbent for the selective extraction of synthetic cathinone, 4-methylmethcathinone, from urine has been successfully prepared and validated.

P88. APPLICATION OF A VALIDATED LC-MS/MS METHOD FOR THE SIMULTANEOUS ANALYSIS OF 9 SYNTHETIC CANNABINOID METABOLITES TO HOSPITAL ADMISSION URINE SAMPLES

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Recently the use of cannabimimetics (more commonly known as synthetic cannabinoids) has increased at an unprecedented rate around the world. As a result the importance for the analysis of synthetic cannabinoids in human matrices is evident and continues to be increasingly challenging since these compounds are constantly being modified and are rapidly becoming available. They have extensive metabolism pathways; therefore the main target compounds in urine specimens are their hydroxyl and carboxy metabolites, which is important to recognise when establishing clinical and forensic toxicology screening and confirmatory methods.
The aim of the study was to apply a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the detection and quantitation of JWH-018 5-OH pentyl, JWH-018 4-OH pentyl, JWH-073 3-OH butyl, AM-2201 4-OH pentyl, JWH-250 4-OH pentyl, JWH-122 5-OH pentyl and JWH-210 5-OH pentyl, and the most recent substances 5-F-AKB48 (N-4 OH pentyl) and 5-F-PB-22 3-carboxyindole in urine samples from individuals admitted to Accident and Emergency, Glasgow Royal Infirmary, UK.
The urine samples were buffered with 1M sodium acetate (pH 5.5) before being hydrolysed with β-glucuronidase. Liquid-liquid extraction (methyl-tert-butyl-ether) was used to isolate and concentrate 9 synthetic cannabinoid metabolites from urine. Chromatographic separation (run-time 11.5 mins) was achieved using a Phenomenex Gemini C18 (150 x 2.0 mm, 5µm) column and a gradient elution system consisting of two mobile phases; 2mM ammonium acetate and 0.1% formic acid in de-ionised water and in methanol. Agilent LC-MS/MS equipped with an electrospray ionisation interface, operated in positive polarity, was used for analysis. Multiple Reaction Monitoring (MRM) mode was used to monitor 2 ion transitions (quantifier and qualifier) for each analyte and 1 transition was used for the internal standard.
All synthetic cannabinoids were detected and quantified without endogenous interferences. The correlation coeffi-

cient (R^2) was ≥ 0.996 for all analytes, with precision < 14% and accuracy ranging from 87 to 110% for all analytes at concentrations of 5, 40 and 75 ng/mL. The limit of detection ranged between 0.01 and 0.25 ng/mL and, the lower limit of quantification for each compound was determined to be 1.0 ng/mL. The recovery ranged from 77 to 108 % and the matrix effects were acceptable, ranging from 81 to 108 % for all analytes at concentrations of 5, 50 and 100 ng/mL. The presented method was successfully applied to 93 authentic urine admission samples collected at Glasgow Royal Infirmary, UK. Four (4.3%) samples were found positive for 5-F-PB-22 3-carboxyindole with concentrations of 6.6, 23.7, 60.3 and 267.7 ng/mL.
A simple, cost-effective and accurate LC-MS/MS method for the simultaneous quantification of 9 synthetic cannabinoid metabolites in urine has been developed and validated. The method has been successfully verified using 93 authentic hospital case samples.

P89. DETERMINATION OF BENZODIAZEPINES, NEW PSYCHOACTIVE SUBSTANCES AND Z-HYPNOTICS IN URINE BY UPLC-MS/MS

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The Norwegian Institute of Public Health annually analyses approximately 7000 urine samples for confirmation and quantification of benzodiazepines and z-hypnotics. The last years several new psychoactive benzodiazepine substances (NPS) have emerged on the illegal market (EMCCDA report march 2015). Although numerous analytical methods for quantification of benzodiazepines and z-hypnotics have been reported, few of these multicomponent methods include several of the new benzodiazepine NPS. An automated high-throughput multicomponent method including the relevant substances is therefore crucial.
The aim of the study was to develop and validate an ultra high performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS) method for the simultaneous quantification of 23 benzodiazepines, new psychoactive benzodiazepine substances (NPS) and z-hypnotics, including metabolites, in 100 µl urine. 7-aminonitrazepam, 7-aminoclonazepam, 7-aminoflunitrazepam, alprazolam, alpha-OH-alprazolam, lorazepam, alpha-OH-midazolam, bromazepam, phenazepam, etizolam, oxazepam, N-desmethyldiazepam, 3-OH-diazepam, OH-flubromazepam, pyrazolam, diclazepam, lormetazepam, delorazepam, zopiclone, zopiclone-N-oxide, 2-amino-5-chloropyridine (ACP) and two zolpidem metabolites were included in the method.
Enzymatic hydrolyses at 60°C for 1 hour was performed before solid phase extraction (SPE) with cation exchange on 96-well plate Plexa PCX columns. SPE was automated on a Tecan robot. Gradient elution was performed on a C18 BEH column (100x2.1 mm, 1.7 µm), with acetonitrile and 5 mM ammonium acetate pH5. 0.5 µl was injected on an Aquity UPLC I-Class system with a Xevo TQS tandem-quadrupole mass spectrometer (Waters). Deuterated analogs were used as internal standards. The stability of zopiclone and its metabolites at different storing conditions was studied in spiked and authentic urine samples.
Linear calibration curves with R^2>0.98 were achieved for all the compounds expect lormetazepam (0.956), zolpidem metabolite 2 (0.957) and ACP (0.965) using a linear calibration curve with 1/x weighting. Within day precision was satisfactory for all the compounds with RSD < 20% except lormetazepam (RSD 25.1%). Validation results including matrix effects, recovery, between day precision, method comparison and stability results will be presented.
The method seems to be suitable for the determination of benzodiazepines, NPS and z-hypnotics in forensic urine cases. To our knowledge, this is the first automated method combining a small sample- and injection volume with the simultaneous determination of 23 benzodiazepines, z-hypnotics and benzodiazepine like NPS. The method will be used for routine forensic analysis for investigations of suspicion of drug intoxications.

P90. DEVELOPMENT OF A QUANTITATIVE POINT OF CONTACT (POC) LATERAL FLOW TEST (LF) FOR OPIATES IN SALIVA

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Existing salivary point of contact tests for drugs of abuse rely on semi-quantitative positive/negative evaluation based on a pre-defined cut off level. Improvements in lateral flow tests reproducibility and recent advances in the quality and sensitivity of lateral flow test readers has widened the opportunity for developing quantitative lateral flow tests. Such tests may help in lowering the level of detection of drugs in saliva thus increasing the window of detection.
The aim of this work is the development of a quantitative point-of-contact test for the detection of opiates in saliva with the aim of extending window of detection
Subjects (n=4) were tested for the presence of opiates in saliva following single and multiple doses of pholcodine linctus cough syrup as detailed. A single 10ml dose of 1mg/mL Pholcodine linctus (Boots pharmaceuticals) was given. Saliva samples were taken immediately before dosing and then at 30 minutes, 1, 2, 4, 6, 24 and 48 hours following dosing. In another experiment, Four subjects were given 10mg pholcodine (10 mL dose of 1.0mg/mL Pholcodine linctus as before) eight hourly for three days (total of 9 doses). A sample was taken immediately preceding the initial dose and then every 24 hours thereafter up to 14 days following the final dose. Saliva samples were collected using the IPRO oral fluid collector device (OFC). The device collects 0.5mL oral fluid using a swab which is then placed in 1.5mL extraction buffer. Following 2 minutes of mixing, 2 drops of the saliva/buffer mixture are added to the opiate LF test cartridge. After 10 minutes incubation, the test line intensity is read using Qiagen LF reader and using a calibration curve, the line intensity is converted to morphine equivalent concentration expressed as

ng/mL oral fluid. The opiate LF test is a competitive immunochromatographic assay using gold labelled anti-opiate antibody.
The opiate LF test has a limit of quantitation for morphine of 5ng/mL. Over the counter pholcodine, in single and multiple doses, was used to represent an occasional and regular user of opiates, respectively. Following a single dose of pholcodine, the oral fluids remained positive (concentration over 5ng/mL morphine equivalent) for at least 48 hours. With multiple dosing subjects, opiates can be detected in the saliva for up to 13 days following the final dosing.
Using recently available sensitive readers of lateral flow test, reliable quantitative point-of-contact tests for drugs of abuse can be developed. In this work, the window of detection of salivary opiates was significantly increased with respect to using semi-quantitative point of contact tests. This approach may help in overcoming one of the main disadvantages of saliva testing, namely, short window of detection. This remains a screening test and as with all screening tests, challenged test results must be confirmed using gold standard procedures (gas chromatography/ mass spectrometry).

P91. COMPREHENSIVE SCREENING ANALYSIS OF MORE THAN 1000 CASEWORK SAMPLES BY USING GC/MS AND UNTARGETED LC/MS/MS ANALYSIS IN PARALLEL

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In clinical and forensic toxicology, detection and identification of a wide range of potentially toxic compounds is of utmost importance. To enable comprehensive screening, the complementary mass spectrometric techniques GC/MS and LC/MS/MS are typically employed.
The aim of the present study was to demonstrate the usefulness of extending a GC/MS-based screening workflow with an untargeted LC/MS/MS technique that was established on a low-resolution, low-budged instrument.
Sample preparation involved a generic SPE procedure employing a mixed-mode material (Spe-ed Scan ABN columns, Applied Separations). Typically, 10 ml of urine or 2 ml of blood/plasma were processed. Cleavage of phase II metabolites part of urine samples was enabled by enzymatic hydrolysis (β-glucuronidase/arylsulfatase, Helix pomatia, Roche Diagnostics). For parallel GC/MS and LC/MS/MS analysis, the SPE eluate was split in two aliquots. LC was performed on a RP-C18 column (Eurospher 100-5 C18, 100 × 2 mm, Knauer). A QTRAP 3200 system (AB Sciex) was used for ESI-MS/MS in positive ion mode under data-dependent acquisition control. A duty cycle included a Q3 MS scan followed by enhanced resolution scans and enhanced product ion scans on the 3 most abundant precursor ions. The intensity threshold for triggering MS/MS experiments was set to 20,000 counts. The obtained MS/MS spectra were matched to the "Wiley Registry of Tandem Mass Spectral Data, MSforID". The GC-MS system consisted of a HP7890 GC device with a HP5975C inert XL mass-selective detector. A DB-XLB column (30 m x 0.25 mm x 0.25 µm, J&W) was used for chromatographic separations. Compound identification was accomplished with the Maurer/Pfleger/Weber 2011 library. Samples were analysed in the native form and after acetylation. All putatively positive identifications were checked by expert reviewing.
More than 1000 authentic samples (blood, plasma, and urine samples) were successfully screened with the presented workflow. The usefulness of analyzing casework samples with a dual system consisting of GC/MS and untargeted LC/MS/MS is exemplified by statistical evaluation of the results obtained from the subset of 506 blood/plasma samples. In this sample set, 629 potentially toxic compounds were detected and confirmed. 55% of compounds were detected with both mass spectrometric methods, 23% of compounds with GC/MS and 22% of compounds with LC/MS/MS only. Due to learning effects, the overall rate of positive identifications obtained with LC/MS/MS increased from 68% in 2012 to 80% in 2014. Compounds preferentially detected with LC/MS/MS included benzodiazepines, beta blockers, and ionic species. GC/MS was favorable for the detection of compounds with low ionization efficiency in ESI(+), such as propofol, diclofenac and ibuprofen. 9% of the compounds identified represented illegal drugs. Other important classes of compounds observed included antidepressants, antipsychotics, hypnotics, sedatives, anesthetics as well as other drugs commonly used in emergency medicine.
A workflow employing GC/MS and untargeted LC/MS/MS analysis in parallel was successfully applied for the comprehensive detection and identification of potentially toxic compounds in >1000 casework samples.

P92. VALIDATION OF A NEW HOMOGENOUS IMMUNOASSAY FOR PREGABALIN TESTING IN URINE SAMPLES

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Pregabalin is a gamma-aminobutyric acid analogue approved for the treatment of epilepsy, neuropathic pain and anxiety disorder in many countries. In addition pregabalin is used in benzodiazepine dependence treatment. Abuse of pregabalin occurs among opiates addicts in germany. However, little is known about the positive prevalence rate in this patient group. This is mainly because pregabalin is not included in the standard urine drug testing panel of most laboratories. We therefore evaluated a new homogenous immunoassay for routine screening.
This study tries to estimate pregabalin abuse in an opiate maintenance therapy patient population and validates an urinary immunoassay at different cutoffs.
Routine urine drug screening samples (n = 1000 ; 752 males, 248 females) from substitution patients were analysed for pregabalin with an UPLC-MS/MS method. The cutoff was set at 10 ng/mL and the internal standard pregabalin-d6 (Cerilliant) was spiked ad 10 ng/mL. Matrix calibration and measuring range was from 1 ng/mL to 1000 ng/mL (13 points). The immunoassay (ARK Diagnostics) was set up on an Olympus AU680 according to the

manufacturer´s instructions. Provided urine calibrators (0, 100, 500, 1000, 2000 ng/mL) were complemented by own urine calibrators at 250 and 750 ng/mL. Calibration was performed prior every series. Pregabalin target values were at 75 and 125 ng/mL for homemade urine controls and the provided controls were at 250 and 750 ng/mL. Cross-reactivity experiments were conducted with 1 mg/ml Gabapentin in 0.9% saline.
UPLC-MS/MS quality control samples with target values at 40.2 ng/mL, 64.0 ng/mL (ACQ Science) and 800 ng/mL (homemade) revealed CVs between 5.4% and 10.5% and mean bias was between 3.5% and 9.2% (n = 72). Intra-assay CV of the immunoassay was below 8% for all four quality control samples (n = 10). Mean bias ranged from -0.9% to 3.7% with CVs between 5.2% and 9.2% (n = 33 series). Cross-reactivity for gabapentin was 0.1% at 1 mg/mL. At the 10 ng/mL UPLC-MS/MS cutoff we found 125 samples positive for pregabalin (12.5%). UPLC-MS/MS cutoff 100 ng/mL: 117 samples were pregabaline positive and 843 samples were negative with UPLC-MS/MS and immunoassay. Only one sample was false negative in the immunoassay. However, 39 samples were false positive (3.9%). In 34 samples (87%) no pregabalin could be detected (<10 ng/mL). Five samples (13%) contained pregabaline between 23 and 70 ng/mL. UPLC-MS/MS cutoff 200 ng/mL: 112 samples were pregabaline positive and 880 samples were negative with UPLC-MS/MS and immunoassay. One sample was false negative in the immunoassay and 7 samples were false positive (0.7%). Two of these samples contained gabapentin. UPLC-MS/MS cutoff 500 ng/mL: 109 samples were pregabaline positive and 886 samples were negative with UPLC-MS/MS and immunoassay. One sample was false negative in the immunoassay and 4 samples were false positive (0.4%).
The pregabalin positive rate was 11.3% (200 ng/mL UPLC-MS/MS cutoff) in the investigated patient population underlining the need for a rapid screening method. At this cutoff nearly all true positives were identified by immunoassay at an acceptable false positive rate.

P93. QUALITATIVE AND QUANTITATIVE ANALYSIS OF FLUOXETINE IN BLOOD AND URINE

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Increasing numbers of cases of poisonings by fluoxetine, either attempted suicide or accidental, combined with the absence of reliable methods for the detection of fluoxetine and quantitation in biological matrices is the basis for the need for the development of new analytical techniques for forensic analysis.
The aim of the present investigation is to use thin-layer chromatography (TLC), UV spectrophotometric and thermodesorption surface - ionization spectroscopy methods for the estimation of fluoxetine in biological fluids.
Took 2 ml of blood (urine of 5 ml) and have finished pH to 8,0-9,0 0,1 N solution NaOH and extracted with 5 ml a ethyl acetate. Chemico-toxicological investigations of fluoxetine have been carried out by TLC, for spectrophotometric estimated of fluoxetine in specimens was used a UV/VIS spectrophotometer (Model 8453, Agilent Technologies, USA) with 1 cm matches quartz cell. Standard solutions of fluoxetine ethanol were prepared with concentrations 1-400 microgram/mL and scanned in spectrum mode between 200-400 nm, thermodesorption surface - ionization spectroscopy. The detection of fluoxetine by TDSIS, both as pure standards and in biological isolates is reported here for the first time.
Method TLC -was recommended. System is ethanol - chloroform - benzene(2:1:2), for detecting: Dragendorf's reactive and etc. Rf 0,59-0,61 (sensitivity 0,5 microgram). Method UV- spectrophotometry can be used for detection fluoxetine in solutions and in extracts from blood and urine the. The spectral characteristic of solutions fluoxetine in ethanol has a light-absorbing maximum at length of a wave of 269 nm. Beer's law was obeyed in the concentration range of 10-300 microgram/mL For the purpose of detection fluoxetine in biological substrat are used method surface - ionization spectroscopy. The thermodesorption range fluoxetine has characteristic peaks at ~96±15 and ~212±10 (sensitivity 0,5 ng). The results successfully have been tested on the blood of patients after oral administration and on the poisoning body blood in overdose cases. An offered technique of isolation, detection and definition fluoxetine approved on an expert material.
The investigations led to the conclusion about the suitability of these methods of isolation, identification and quantitative determination of fluoxetine in biological fluids. The results of the given investigation have been introduced into practice of all forensic-chemical and medical laboratories of the Republic of Uzbekistan.

P94. INVESTIGATING THE ENHANCEMENT IN SELECTIVITY FOR THE ANALYSIS OF METHYLDIENOLONE IN URINE SAMPLES BY DIFFERENTIAL MOBILITY SPECTROMETRY

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One of the most common issues in LC-MS/MS method development is the presence of matrix isobaric interferences that makes integrating chromatographic peaks and reaching required detection levels challenging. Commonly these interferences are removed by either adjusting HPLC conditions or by modifying sample preparation. These approaches however increase both method development time and the final method result turnaround times. Differential mobility spectrometry (DMS) has the potential to resolve these interfering compounds during the LC-MS/MS run enabling minimal sample preparation and reduced LC run times. Here we investigated the use of an ion mobility cell to resolve isobaric interferences in the analysis of methyldienolone in human urine samples that cannot be achieved by tandem mass spectrometry alone.
We aimed to investigate the capabilities of a DMS device coupled with LC-MS/MS to provide a highly selective quantitation method for methyldienolone; separating matrix isobaric interferences from the target analyte, in a simple dilute and shoot workflow.

The SCIEX QTRAP® 5500 was operated in Multiple Reaction Monitoring (MRM) mode. Electrospray Ionization is employed in positive polarity. Three selective MRM transitions (237.2 / 135.1; 237.2 / 91.0; 237.2/ 77.0) were monitored for methyldienolone. Elution is by gradient through Waters C18 column maintained at 60 °C at a flow rate of 500 µL/min. The injection volume was set to 10 uL. DMS device was operated at low temperature and low resolution setting without the use of modifier. Separation voltage and compensation voltage were set at 3000 V and 3.0 V respectively while the offset voltage was kept as -3.0 V. Urine samples were centrifuged, diluted 5 times and analysed as per the dilute and shoot methodology.
With dilute and shoot LC-MS/MS method based on MRM, isobaric interferences were found at the retention time of Methyldienolone. Also, there was high baseline with MRM transitions used for this analysis. By using the LC-DMS-MS/MS setup and selecting the appropriate value for compensation voltage, all isobaric interferences were selectively resolved from the LC-MS/MS methyldienolone chromatographical peak and the high baseline completely removed. Matrix matched calibration curve was generated from 0.1 ng/mL to 500 ng/mL. Regression co-efficient obtained for the linear regression plot is (r): 0.9956 with weighting factor $1/X^2$. Spiked concentration of 0.5 ng/mL of urine sample (1pg on column) was selected as LOQ with S/N> 70 with % CV=2.18. Reproducibility of the area at LOQ level (n=20)gave % CV: 3.26%.
The DMS technology was demonstrated to be successful in the optimization of a dilute and shoot methodology for the analysis of methyldienelone; allowing the use of this simplified sample preparation approach by eliminating all interfering ions.

P95. EVIDENCE OF CHRONIC ALCOHOL CONSUMPTION BY AN ANAESTHESIOLOGIST AS DOCUMENTED BY HAIR ANALYSIS

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Chemical dependency is a disease that can affect all professions. Among the health care professionals, anesthesiologists represent a specific group. Opioids are the drugs of choice but alcohol is mostly abused by older anaesthesiologists. Complementary results can be obtained with usual markers of excessive alcohol drinking, such as blood carbohydrate deficient transferrin (CDT) or liver function tests (LFT as GGT) and major markers of ethanol consumption in hair like ethyl glucuronide (EtG) and fatty acid ethyl esters (FAEEs).. However, given the legal consequences, it must be documented under the best conditions. This issue was recently observed during the prosecution of an anaesthesiologist.
A 45-year old woman, anaesthesiologist, involved in the death of a patient during surgery while impaired with alcohol, was asked to provide blood and hair samples to document her history of excessive alcohol consumption.
The aim of this report is to present the biological strategy and the associated pitfalls observed during the toxicological investigations.
All analyses were achieved under ISO17025 accreditation. Blood and hair samples were collected 6 weeks after the incidence while the anaesthesiologist was in jail for 5 weeks EtG was tested by liquid chromatography – tandem mass spectrometry after solid-phase extraction and separation on a hydrophilic interaction liquid chromatography column (HILIC). For the determination of FAEEs, a procedure based on headspace solid-phase microextraction, followed by gas chromatography – mass spectrometry was applied. Blood and liver function tests (ĐGT) were achieved by classic haematology and biochemistry assays.
Used cut-off concentrations related to chronic excessive alcohol consumption in hair are respectively 30 pg/mg for EtG and 1.0 ng/mg for total FAEEs. Concentrations in submitted hair (0 - 6 cm segment) were tested positive at 59 pg/mg for ETG and at 1.25 ng/mg for total FAEEs. The CDT result level in the blood sample provided by the subject was 1.3 %, which falls within the range of a healthy individual (< 1,7 %) and supports the hypothesis that up to 4-6 weeks prior to sample collection alcohol had not been chronically and excessively consumed. The LFT result for the blood sample provided by the subject showed a GGT reading of 33 IU/L and was within the range of a healthy adult (< 45 IU/L), suggesting no liver damage consistent with long term alcohol abuse.
The results of the hair tests have confirmed chronic excessive alcohol consumption of the anaesthesiologist to alcohol. Obviously hair and blood tests do not have the same periods of detection. The findings of positive EtG and FAEE hair results with negative blood tests have been explained by the fact that the blood was collected several weeks after the event, once the anaesthesiologist was in jail.

P96. POTENTIAL TOXIC LEVELS OF CYANIDE AND CYANOGENIC COMPOUNDS IN ALGERIAN BITTER ALMONDS AND APRICOT KERNELS

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The toxicity of apricot kernels and bitter almonds is related to the release of cyanide from cyanogenic compounds, which is responsible for much of the neurological, cardiac and respiratory deterioration. The intoxications with cyanogenic glycosides listed in the poisons center of Algiers (Algeria) occupy a significant proportion of plant poisoning (6%). The identification and quantification of cyanide and cyanogenic glycosides is an important source of information when evaluating the toxicity of these plants.
This report presents a primary study to determine the level of cyanide and cyanogenic compounds and their major effective ingredient, amygdalin, in bitter almonds and apricot kernels collected in various regions of Algeria.An analytical and clinical strategy is proposed for the diagnosis and management of poisoning caused by these plants.

30 samples were collected in June and July 2011 in different regions of Algeria. Weight and height were measured. After methanolic extraction of bitter almonds and apricot kernels, cyanogenic compounds were identified and quantified by HPTLC whereas amygdaline was quantified by HPLC-MS/MS. In parallel, cyanide was liberated by endogenous enzymatic hydrolysis and analyzed by HS-GC-FID
The average weight Bitter almonds and apricot kernels were respectively (0.96 g, 0.49g) and the their medium size were respectively (2.13 cm, 1.6cm). Three chromatographic methods (HPTLC, HPLC-MS/MS, and GC-FID HS) were developed and validated for assessing toxicity of plants with cyanogenic compounds. Analysis of amygdalin by HPLC MS/MS revealed rates ranging from 100 to 8000 mg/kg (0.01 -0.8%) for bitter almonds and 200 to 5500 mg/kg (0.02-0.55%) for apricot kernels. All cyanogenic glycosides measured by HPTLC have given high values compared to HPLC-MS-MS. The hydrogen cyanide rate have varied from 182 to 4146 ppm (m = 1372 ppm) for bitter almonds and 383 to 2774 ppm (m = 1533 ppm) for the apricot kernels.Cyanide and amygdalin levels were similar to the data found in the literature. The results showed that cyanogenic glycosides amounts correlate with cyanide released (R= 0.953, n=16), but there were no good correlation with amygdalin amounts. It suggests the presence of other cyanogenic compounds excluding amygdalin.
Based on the lethal dose of cyanide to humans and cyanide levels in these plants, the number that could be responsible for a fatal poisoning varies from 11 to 21 bitter almonds (mean = 16) and from 25 to 211 apricot kernels (mean = 99).

P97. OXIDATIVE STRESS EFFECT OF HEROIN AND CANNABIS ADDICTION IN EGYPTIAN POPULATION

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Addiction is one of the world's major health problems with large direct health costs (psychiatric and physical).
The present study aimed to investigate the oxidative stress effects of heroin and cannabis addiction and their impact on the immune system.
Eighty four individuals include 60 addicts and twenty four control subjects; their ages ranged from 18 to 38 years were studied. The exclusion criteria were subjects who had diabetes, history of liver disease, unstable cardiovascular, peripheral vascular, respiratory, or gastrointestinal disease or malignancy. All addicts and control subjects were selected from heavy smokers persons to neglect the effect of nicotine smoking when comparing between addict and control groups. Moreover, all addict subjects were selected from the chronic ones (addict for more than 6 years). From each subject ten ml urine samples were screened for detection of substance abuse. In addition four ml blood was collected from each subject for detection of both cytokines; interleukins (IL2 & IL6) and reactive oxygen species (ROS) (reduced glutathione; thiobarbituric acid reactive substances {TBARS} and protein oxidation) after taking informed consent.
The results revealed a significant decrease ($p < 0.001$) in serum IL-2 in males & females heroin and cannabis addicts (64.9 + 2.99 pg/mL & 62.93 + 2.19 pg/mL respectively) and (64.45 + 1.70 pg/mL & 61.67 + 2.18 pg/mL respectively). While there is no significant difference in the IL-6 level. Regarding ROS; there is significant decrease ($p < 0.001$) in protein thiols level in both heroin and cannabis males & females addicts compared to control group (418.8 + 13.10 µmol/L & 406.8 + 14.99 µmol/L respectively) and (412.9 + 14.35 µmol/L & 408.8 + 14.67 µmol/L respectively). While, there are significant increases ($p < 0.001$) in both TBARS (0.35 + 0.01 mmol/dL & 0.32 + 0.01 mmol/dL respectively) and (0.32 + 0.01 mmol/dL & 0.31 + 0.01 mmol/dL respectively) and protein oxidation (121.7 + 6.27 µmol/L & 116.6 + 5.02 µmol/L respectively) and (125.0 + 8.04 µmol/L & 119.8 + 7.52 µmol/L respectively) in heroin and cannabis male and female addicts in comparison to the control group.
It could be concluded that heroin and cannabis might play a role in the immunotoxicity through their effects on the ROS.

P98. CASE REPORT: SERUM AND CSF CRIZOTINIB DETERMINATION IN TWO ALK-POSITIVE NON-SMALL CELL LUNG CANCER PATIENTS WITH CNS METASTASES BY LIQUID CHROMATOGRAPHY ELECTROSPRAY IONIZATION-TANDEM MASS SPECTROMETRY (LC-ESI-MS/MS).

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Crizotinib ($C_{21}H_{22}Cl_2FN_5O$, MW 449.12) is an anaplastic lymphoma kinase (ALK) tyrosine kinase inhibitor (TKI)3 that leads to responses in most patients with NSCLC (non-small cell lung cancer) harboring ALK translocations. Crizotinib showed promising results in the treatment of NSCLC patients with ALK translocations and therefore received accelerated approval for ALK positive NSCLC in August 2011 by the FDA. Due to its relatively recent introduction into pharmacological practice, very few analytical methods are available in literature for crizotinib determination so its therapeutical monitoring (TDM) is hampered and in-vivo metabolic studies limited.
Here we report on a recently published LC-ESI–MS/MS method to easily and successfully determine crizotinib in human serum and CSF and its application to two patients with CNS metastases from ALK-positive NSCLC who experienced clinical benefit from crizotinib treatment.
The analytical system used was an Agilent UHPLC 1290 Infinity coupled with an Agilent 6460 triple-quadrupole via Jet Stream Ionization (JSI) source. The composition of the mobile phases was: 5 mM ammonium formate in water

containing 0.1% of formic acid and acetonitrile added of 0.1% formic acid. Chromatographic separations were carried out in a 2.1 × 100 mm, 1.8 µm Zorbax Eclipse Plus PhenylHexyl column, thermostated at 50 °C, in a gradient elution mode at a flow 0.2 mL min-1. Source parameters were optimized on a crizotinib standard solution by syringe infusion technique. The monitored transitions were m/z 450-->367, 450-->260.1, 450-->177, 450-->84.1. Samples were precipitated in solvent and diluted; finally 5ul were injected in the LC-MS/MS system. The limit of quantification (LOQ) for the developed method was 0.1 ng/ml. Linearity was verified in the range 0.1-200 ng/ml. With the aim to minimize matrix effect, external standard addition method was adopted to quantify the analyte in real samples. The developed method allowed for the successfully detection and quantification of crizotinib in two real cases. The Case 1 proved a plasma concentration for crizotinib of 587 ng/mL and a CSF concentration of 0.35 ng/mL (CSF-to-plasma ratio of 0.0006), whereas Case 2 showed a concentration of 800 ng/mL and 0.80 ng/mL (CSF-to-plasma ratio of 0.001) for plasma and CSF, respectively.
The cases presented herein are remarkable for a few reasons. First, they confirm poor CSF concentrations for crizotinib. This finding, which is in line with what has been reported by Costa et al., confirms the poor ability of crizotinib to cross the BBB (blood-brain barrier). Secondly, they show that the CSF-to-plasma ratio of crizotinib in patients with CNS metastases from ALK-positive NSCLC may vary considerably depending on type of CNS disease and prior treatment for CNS metastases.

P99. THERAPEUTIC DRUG MONITORING OF MEROPENEM IN HUMAN PLASMA BY LC-MS/MS

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Meropenem is an effective beta-lactam antibiotic, frequently used to treat serious infections including meningitis and pneumonia. The therapeutic drug monitoring of meropenem is important because plasma concentrations have to be maintained above the minimum inhibitory concentrations (MIC).
The aim of this study was the development and validation of a sensitive and accurate liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the determination of meropenem in human plasma.
Blood samples were transported to the Laboratory in ice, centrifuged and the plasma samples were stabilized by adding MOPS and were stored at -80°C until analysis. Individual stock solutions of meropenem and ertapenem (internal standard) (1 mg/mL) were prepared in MOPS and working solutions for calibration and controls were prepared from the stock solution by dilution using MOPS. Meropenem calibration standards and quality controls were prepared by spiking blank human plasma with appropriate amounts of the working solutions. All samples (calibration standards, quality controls and clinical samples) were thawed and 150 µL of sample were mixed with the internal standard and buffered with acetate at pH 4.0. An aliquot was transferred to a Microcon TM YM10 and centrifuged at 15,000g for 20 min, then 200 µL of the filtrate were transferred to an auto-sampler vial and 5µL were injected into the LC-MS system. Gradient elution was performed by a Restek Allure PFP Propyl column and electrospray source (ESI) in the positive ionization mode was applied and multiple reaction monitoring (MRM) mode was used for the quantification.
The method was linear from 0.1 to 40 µg/mL (R^2 =0.99857), selectivity was checked by analyzing 25 blank human plasma, the limit of quantification (LOQ) was 0.5 µg/mL and the accuracy ranged from 82 to 100% at three concentration levels (8, 24 e 40 µg/mL). The precision of the method was determined calculating CV at three concentration levels (8, 24 e 40 µg/mL) for 10 times in the same day (intra-assay precision) and one time for 10 days (inter-assay precision). Intra-assay precision ranged from 3.6 to 4.7% at each level and the inter-assay precision from 4.1 to 5.0% After the method validation, the assay was applied to blood samples from patients obtained in two ways: before and 30 min after infusion of meropenem: in the first group the concentrations ranged from 1.7 to 14.9 µg/mL, and in the second from 18.1 to 82.3 µg/mL.
The proposed method is very sensitive, rapid, specific and accurate, and was successfully applied to therapeutic drug monitoring of meropenem in clinical routine applications.

P100. A HIGH PREVALENCE FOR CRACK SMOKING AS REVEALED BY ROUTINE URINE DRUG TESTING

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Drug users who smoke crack cocaine (free-base cocaine) belong to a marginalized sub-group exposed to severe health risks and great social harm. Smoking crack cocaine lead to formation and inhalation of a pyrolytic degradation product, methylecgonidine (MED), which is considered to be a highly specific biomarker for the smoking route of administration. MED is not stable in biological samples, but prone to chemical and enzymatic hydrolysis forming a stable metabolite, ecgonidine (ED). Except for a few dedicated studies, the utility of MED and ED in routine analysis has been largely neglected.
The purpose of the study was (1) to estimate the proportion of crack cocaine smokers by laboratory analysis in a population of drug users for whom routine drugs-of-abuse testing was requested; (2) to establish a LC-MS/MS method for routine determination of MED and ED in order to facilitate an early recognition of a more severe use of cocaine compared to the snorting route of administration.
A new LC-MS/MS method was validated for quantitative analysis of a number of relevant cocaine me-tabolites in urine. Anonymized study samples (n=110), pre-screened positive for benzoylecgonine were obtained from various clinical biochemistry laboratories and prison institutions in Denmark. Limit of quantification for ED and MED was 30

ng/mL. Compound identification was based on multiple ion monitoring of three ions/compound.
Eighty-four samples (76.4%) contained ED and/or MED, thereby conclusively found positive for crack cocaine smoking. MED was only detected in 5.9% of these positive samples. The study shows a high prevalence of crack cocaine smokers in a group of cocaine users, where drug use has become problematic. The prevalence is three-fold higher than a recent estimate of the proportion of crack cocaine smokers in treatment made by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA).
Confirmative analytical methods for cocaine used in clinical toxicology may be more informative if urinary biomarkers for crack cocaine smoking are included. This may lead to an early recognition of severe drug use and potentially have influence on treatment and outcome. This additional test has minor influence on cost-of-analysis. We advocate that MED and ED, as specific biomarkers for crack cocaine smoking, should be given more attention in routine analysis, proficiency testing and post-analysis interpretation of results.

P101. EVALUATION OF AMPHETAMINE TYPE STIMULANTS (ATS) HAIR/URINE RESULTS FOR PROBATION SYSTEM IN TURKEY

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International attention is focusing more and more on the growing issue of amphetamine-type stimulants (ATS). Particularly over the last 10 to 15 years, abuse of ATS, involving amphetamines (amphetamine and methamphetamine) and substances of the "ecstasy"-group (MDMA, MDA, MDEA, etc.), is a growing problem in Turkey therefore detection of these substances analytical approaches are important for probationers. The widespread of individual and social problems dependent on substance use, provoked the new legislative approaches in Turkey. In 5272 numbered Turkish Penalty Code's 109th paragraph it is suggested that probation measures which is related to "The evaluation according to a psychiatrist clinical diagnosis with laboratory findings for therapies of narcotic, stimulant or inhalant substances and alcohol addiction" put into practice in June 2005. According to this law, treatment programme is applied for six weeks and probationers are required to provide urine specimens as a condition of their release once in two weeks.
The aim of this study is evaluate urine specimen screening results 'effectiveness with hair analysis results by GC-MS.
The study was developed in BATI institute and hair samples were obtained from probationers. Firstly, probationers' urine samples were screened for drugs using Cloned Enzyme Donor Immunoassay (CEDIA) and then hair samples were taken from amphetamine and ecstasy positive cases in the last week of treatment programme with voluntary consent form (Ethics committee approval was obtained). These samples were generally cut as close as possible to the skin from the posterior vertex. Total length was approximately 1.5 cm. Then, hair sample was extracted with simple and fast liquid-liquid extraction procedure. Samples solutions were injected into the GC–MS. After analysis we evaluated urine test results with hair analysis results.
For monitoring substance abuse, urine is preferred (legally), with this study hair samples were analyzed by GC-MS. MDMA (3,4-methylenedioxy-methamphetamine) were detected in hair samples with LLE by GC-MS. All urine results (n=25) were negative and therefore affirmative report is given (successfully probation). In contrast to urinanalysis, hair tests revealed consumption in % 20 cases for ATS.
Probationers knew which day(s) of the week they would be required to provide urine specimens. This gap, combined with knowing when urine specimens are required, means that probationers can try to abstain from drug use to avoid detection. In our study at least one of three urine analysis was negative in analysis period. These results can make conflicts for clinician (physician) when evaluate patients' clinical outcome with urine test results. In this study, % 20 of cases, which tested urine "negative", and hair "positive" for ATS is indicative for the assays' failure to detect ATS in urine. This study shows that for the interpretation of laboratory results, hair analysis is supportive for the physician in addition to urine analysis.

P102. MERCURY DETERMINATION IN WHOLE BLOOD

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Due to the toxic effects of mercury on human organism is necessary to obtain information about mercury level in the body within a reasonable time and quality. The method for the routine determination of the mercury content in whole blood is described. The measuring range is from 1 to 10000 nmol/L.
The aim of the present study was to evaluate and and put into a routine practise an appropriate method for mercury level determination in biological fluids. A single-purpose device AMA based on AAS principle has been chosen for this task.
Triplicate of 100 ul blood sample is required for measurement on AMA 254 mercury analyzer. Sample is burned and mercury vapour is captured by an amalgamator. Second heating release mercury vapour to cell for AAS measurement. Atomic spectrometer is an integrated part of the device.
There were measured 1171 whole blood patient samples in the period from October 2012 to March 2015 in core laboratory SPADIA Ostrava. For men, there was among 630 entities with mercury value mean 9.1 nmol/L and SD of the mean 8.7 nmol/L. Regarding women there was 541 subjects tested and the mercury value mean was 6.9 nmol/L, SD 6.5 nmol/L. The mean of mercury value for 20 years younger patients (58 people) was 3.4 nmol/L. The mean for rest of patients (older than 20 years) was 8.4 nmol/L. Seven male persons exceeded 50 nmol/L toxic cut-off for mercury.

Poster abstracts

The high mercury level was repeatedly (four times) found in one 41 years old man – 67.3 nmol/L, SD 15.2 nmol/L. The reason was probably his specific diet based on daily consumption of fish.
An AAS method for determination of mercury content in whole blood has been validated and successfully applied to real samples.

P103. MMB-CHMINACA BLOOD CONCENTRATIONS IN RECREATIONAL USERS AND FATAL INTOXICATIONS OVERLAP

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MMB-CHMINACA (methyl N-{[1-(cyclohexylmethyl)-1H-indol-3-yl]carbonyl}-3-ethyl-L-valinate) is one of 30 new synthetic cannabinoids (SC) reported within EU in 2014. The easy accessibility of SCs attracts especially young people; 40% of the SC-related contacts directed to the Swedish Poison Center concerned a person less than 20 years. The adverse effects of SCs can be severe, including unconsciousness, acute psychosis, renal failure, and cerebral or cardial ischemia. The legislation runs constantly behind and as one compound is scheduled as an illegal drug, the suppliers provide a new structural modification. The drug testing laboratories need to be able to rapidly react to these changes and develop selective and sensitive methods for the detection of SCs.
The aim of this study was to develop a liquid chromatography-tandem mass spectrometry (LC-MS-MS) method for the quantification of MMB-CHMINACA in whole blood, and apply it to both antemortem and postmortem cases. To 0.5 gram of blood 1.0 mL of acetonitril/ethanol (90:10) with 0.075% formic acid and 0.025 mL of internal standard were added, the sample was mixed and centrifuged, and the aliquot was transferred to a vial. MMB-CHMINACA was quantitated in positive electrospray ionization mode using an Agilent 6460 triple quadrupole instrument with a 1290 Infinity UHPLC. Gradient elution from 5% B to 95% B over 5.2 minutes with a Zorbax Eclipse Plus C18 (2.1×50mm, 1.8 µm) column was used. Phase A was 0.05% formic acid in 10 mM ammonium formate and phase B was 0.05 % formic acid in methanol. The flow rate was 0.6 mL/min, and the injection volume was 4 µL. For MMB-CHMINACA, two transitions m/z 385/240 and m/z 385/144 were measured. Quantitation was done with the internal standard compensation using AM-2201-d5 (m/z 366/127).
Validation included selectivity, calibration model, accuracy and imprecision, and the method was applied to the whole blood samples from 29 recreational users and 6 autopsy cases that were screened positive for MMB-CHMINACA using LC-time-of-flight MS. Interfering peaks co-eluting with the analyte were not detected in ten negative antemortem and ten negative postmortem cases. The method was linear from 0.0001 to 0.070 µg/g but in routine, the ULOQ was lowered to 0.050 µg/g to reduce the number of calibrators. Between-day imprecisions at 0.0002, 0.001, and 0.035 µg/g were 2%, 4%, and 5%, respectively, and the corresponding accuracies were 108%, 106%, and 112%. The concentration of MMB-CHMINACA in 29 recreational users ranged from 0.0002 to 0.019 µg/g (mean 0.005, median 0.003 µg/g). The concentrations in 6 autopsy cases ranged from 0.001 to 0.010 µg/g (mean and median 0.004 µg/g), and thus overlap with the recreational users.
Two autopsy cases were signed out as fatal intoxications with MMB-CHMINACA, which was the only positive finding at 0.001 and 0.003 µg/g in femoral blood. The autopsy findings were unremarkable, nevertheless, the circumstances pointed towards MMB-CHMINACA as the cause of death, since both deceased had smoked SCs prior to death. We conclude that MMB-CHMINACA causes acute side-effects that may be fatal, however their mechanism is unknown.

P104. CHEMICAL PROFILE OF VOLATILE ORGANIC COMPOUNDS (VOC'S) USED AS INHALANTS SEIZED AT THE BRAZILIAN CARNIVAL

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The terms "inhalants" and Volatile Organic Compounds (VOCs) are used to describe a wide range of volatile chemicals that may be inhaled accidentally or intentionally. The extensive availability and low cost of inhalants have contributed to an increased incidence of intentional inhalation of volatile substances during popular festivals in Brazil. During the Carnival of Bahia, in 2015, more than 60 glass ampoules and bottles similar to those containing perfumes were seized by local police. According to the National Agency of Sanitary Surveillance (ANVISA), the marketing and the recreational use of these substances is illegal under Resolution 344/98 which lists the prohibited substances. However, some narcotic substances that are not prescribed are being illegally used in an attempt to circumvent the law.
The objective of this work is to present the chemical profile of inhalants seized during the Carnival of Bahia in the year of 2015 and the occurrence of new volatile substances used as drugs of abuse that are not proscribed in accordance with brazilian law.
Aliquots of 500 uL of fluid were collected from each glass vial through the original spray bottles and transferred to the headspace vials (20 mL). The samples were injected (2.5 mL) and analyzed by HS-GC-MS in order to carry out screening and identifying volatiles compounds such as ethanol, ethyl chloride, methylene chloride, diethyl ether and others fluorinated and chlorinated organic solvents commonly have arisen in such samples.
61 samples were analyzed and 13 different volatile organic compounds were identified, with prevalence of halogenated substances and more than one volatile compound per sample. Prevalence of the identified compounds were as follows: Ethanol 45,9% (n=28); Hexane 1,6% (n=1); Diethyl ether 3,3% (n=2); Dimethyl ether 16,4% (n=10); Ethyl chloride 6,5% (n=4); Methylene chloride 75,4% (n=46); Chloroform 30% (n=18); Dichlorofluoroethane 41% (n=25); Difluorochloromethane 5% (n=3); Chloromethane 3,3% (n=2); Dichloroethene 1,6% (n=1);

It was observed the prevalence of halogenated substances containing fluorine and chlorine, especially methylene chloride and dichlorofluoroethane used as refrigerant (called HCFC-141b). The latter substance appears in 41% of the samples and it is not illegal under Brazilian law, featuring a major concern for health authorities, due to their cause cardiac arrhythmia in pontencial and depression of the central nervous system (CNS).

P105. THE EFFECT OF ADULTERANT AGENTS ON URINARY SCREENING TEST

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The subjects may try to mask the drug use(through specimen tampering)and therefore a false-negative test results may occur which is very important for probationers.Tampering typically takes one of three forms:urine dilution (flushing),substitution or adulteration.Drug testing by screening methods can be invalidated with the use of some in vitro adulterants.The drug metabolites can be chemically converted to a compound which will not be able to be recognized by the antibody of the immunoassay.Additives interference to the laboratory test or destroy the drugs in the urine.Some of the most popular used goods are household products,such as laundry bleach,table salt,toilet bowl cleaner,hand soap and vinegar etc.Several spot tests have been described to detect the presence of such adulterants in urine.
In the study,we want to evaluate some household adulterants effects on qualitative on urinary screening test systems.
As a household adulterants we used; liquid bleach (%4.5 sodium hypochlorite),apple vinegar,eye drop(0.1 mg benzalkonium chloride) and also drain opener (%90±2 NaOH, %10±2 Na2CO3).The urine pool samples were selected from probationers who were reported 11-nor-9-Carboxy-THC(THC-COOH), Amphetamine (AMP) and 3,4-methylenedioxy-methamphetamine (MDMA) positive results submitted to BATI laboratory for drugs of abuse analysis (THC-COOH adjusted 58 ng/ml,AMP adjusted 1261 ng/ml,MDMA adjusted 511 ng/ml).For adulteration 5 ml urine was taken, bleach concentrations prepared in 50% (v/v) and used 10,100 and 1000 µL,vinegar concentrations prepared in 50%(v/v)and 100%(v/v) used 100 and 1000µL, eye drop used 1,2 and 10 drop,drain opener was weighed 10,100 and 1000mg.Each sample analysed two times (n=26) urine adulteration test stript (Intect 7), Dipcards(QuickTox Drug Screen and Euphratest), immunoassay (CEDIA,MGC 240) and confirmated by GC/MS. The mean concentration were evaluated, also physical characteristics of adulterated urine samples were recorded.
Physical characteristics of adulterated urines(appearance and smell) observed subjectively,7.7% dark yellow,strong bleach and frothy,7.7% mild vinegar,23% cloudy,suspension and undissolved powder,7.7% cloudy.Urine adulteration test stripts(Intect 7) show that abnormal results for pH 15.8%,for glutaraldehyde 7.7%, for specific gravity 61.25%,for bleach 7.7%,for pyridinium chlorochromate 7.7% respectively. Immunoassay results; 10-100 µL bleach add. make AMP level higher than 5000 ng/ml,1000 µL bleach add. THC-COOH and AMP could not detected. Vinegar and eye drops did not make any differences. Drain opener did not make any difference for THC-COOH,but low amount of drain opener for(10 mg) was observed to increase AMP.Both dipcards show negative results for AMP (false-negative).For THC-COOH results,QuickTox Drug Screen were 35,8% negative and Euphratest were 23,1% negative (false-negative).
It is demonstrated that evaluation of integrity of urine specimens is important for probationers drug monitoring. Especially qualitative dipcards give false-negative results. But, it is should be point out semi-quantitative immunoassay systems Sample Check part it is not sufficient for low level adulteration, therefore urine adulteration test systems should be taken much more attention.

P106. METHOD VALIDATION AND APPLICATION OF A LIQUID CHROMATOGRAPHY-HIGH RESOLUTION MASS SPECTROMETRY METHOD FOR DRUGS AND PLANT ALKALOIDS IN URINE

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Immunochemical screening is widely used for drugs of abuse in urine but covers a limited number of analytes. Mass spectrometric methods can be used for larger number of substances to analyze and produce reliable identifications.
The potential of high-resolution MS screening technique is to cover practically unlimited number of analytes for which reference material are still lacking, e.g. internet drugs, makes it attractive for application also in clinical screening for drugs of abuse. The present work was aimed at developing and validating a liquid chromatography-high resolution mass spectrometry method for drugs and plant alkaloids analysis in urine using an LC–Q-Exactive mass spectrometer (Thermo Scientific Co.) and compare it with LC-tandem MS.
Analytes were: hydromorphone, dihydrocodeine, pregabaline, hydrocodone, ketamine, petidin, meprobamate, dextrometorphane, mescaline, psilocyne, lysergic acid amide, scopolamine, harmaline, atropine, yohimbine, mitraphylline, ibogaine, mitragynine, harmine. The sample preparation consisted of mixing 50 µL urine and 200 µL of deuterium-labelled petidine in water. A rapid chromatographic gradient profile at a flow rate of 0.5 mL/min with a total run time of 6 min on a 100 mm Hydrosphere C18 column (YMC Co Ltd) preceded by a pre-column was used. Instrumental MS features were positive electrospray ionisation, full scan in the mass range between m/z 100 – 650 (positive mode) and resolution power was set to 70000. Identification criteria were correct retention time and exact mass of protonated molecule. 278 randomly selected patient urine samples were analyzed with both triple mass spectrometer (SRM) and LC-HRMS techniques
The measuring range in the LC–HRMS method was 50-2000 ng/mL and the reporting limit was set to 100 ng/mL. The

intra- and inter-assay imprecision for low control (100 ng/mL), for middle control (500 ng/mL) and for high control (1500 ng/mL), expressed as the coefficient of variation was below 15% for all compounds (N=15). Some influence (-18% to 30 %) on the ionisation response from urine matrix was noticed in the experiment using standard addition to urine samples and to reference water solutions. No interference was observed analyzing 42 negative authentic patient urine samples (< 25 ng/mL). The number of false positive and negative findings for all compounds (reporting limit was set to 100 ng/mL) with LC-HRMS technique was < 0.7 % and 0%, respectively. The false positive results were only observed close to the reporting limit and were related to uncertainty in quantification.
The LC-HRMS method enabled identification and quantification of drugs in urine and was applied in routine use.

P107. ULTRA-FAST SEPARATION AND QUANTIFICATION OF ISOBARIC BARBITURATES IN SERUM USING LDTD-MS/MS COMBINED WITH DIFFERENTIAL MOBILITY SPECTROMETRY

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Although the medical use of barbiturates has declined since the 1970s, and street abuse was also in decline, high school surveys suggest abuse has been rising over last 10 years. A common reason to abuse barbiturates is to counteract the symptoms of other stimulating drugs such as cocaine and methamphetamines. Barbiturates are also commonly used in suicide attempts. Analytical methods must not only be accurate, but also highly specific and rapid to identify provenance of street drugs.
Amobarbital and pentobarbital differ in the position of a methyl group; therefore a long chromatography is generally required to obtain sufficient resolution on a LC-MS/MS. Ultra-Fast LDTD (Laser Diode Thermal Desorption) ion source combined with a mass spectrometer equipped with differential mobility spectrometry is evaluated to achieve an accurate, specific and reproducible analytical method. A small volume of extracted sample is deposited into LDTD analyzing plate and dried. The sample is thermally desorbed in few seconds and the vapor ionized by APCI prior entering the mass spectrometer. As there is no chromatography, ion mobility is used for isomers separation.
25 µL of serum is diluted with 50 µL of internal standard (5 µg/mL phenobarbital-d5 in methanol:HCl (0.1N) / 25:75) and extracted with 100 µL Ethyl Acetate. After vortexing, 2 µL of upper layer is spotted onto 96-well plates and evaporated to dryness. The mass spectrometer is operated in negative MRM mode with COV values of 6.8 and 5.8 for amobarbital and pentobarbital respectively. Water is used as modifier in DMS. Transitions monitored are 225/42 for amobarbital-pentobarbital and 236/42 for phenobarbital-d5. Run time is 9 seconds sample to sample.
DMS optimization includes modifiers (water, methanol, acetonitrile, 2-propanol), Separation Voltages (SV) and Compensation Voltages (COV). Best results are obtained using water as a modifier with a SV of 4300. Optimal separation is achieved when COV values of 6.8 and 5.8 are used for amobarbital and pentobarbital, respectively. Calibration curve and QCs were prepared using blank serum samples. Excellent linearity (R^2 > 0.997) with no sign of carryover within the quantification range (125 to 25000 ng/mL). Blank interference is less than 10% at the LLOQ. Intra-run accuracy is between 85.3 and 116.3 % and precision is between 1.5 and 15.0 %. Method specificity is tested using three levels of QC containing individual drug. At highest level of amobarbital QC, we observed 1.2% contribution to Pentobarbital transition. Conversely, no signal at amobarbital transition is observed running Pentobarbital high QC. Accuracy ranging from 85.2 to 119.6 % and precision between 2.5 and 17.6 % are obtained. Method robustness was evaluated with success by variation of HCl concentration from 0.05 to 0.15 N, test of 6 different serum lots and 10 common drugs spiked as QC including cocaine and methamphetamine.
Development and validation of a specific quantification method for isobaric barbiturate drugs in serum using LDTD-DMS-MS/MS at 9 seconds sample to sample.

P108. A LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY METHOD FOR THE DETERMINATION OF NINE ANTIDEPRESSANTS IN SERUM: IMPORTANCE OF CHROMATOGRAPHY

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Tricyclic antidepressants (TCA) are used in various psychiatric disorders and pain management. With narrow therapeutic windows, a high risk of cardiotoxicity and central nervous system toxicity, and a large variability in concentrations achieved based on the dosage, drug quantitation is performed in both clinical (therapeutic drug monitoring) and forensic cases. Traditionally, the quantitative analysis of TCAs in serum has used high performance liquid chromatography (HPLC) which can have interferences from other medications.
The aim of this study was to validate a liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the quantification of nine TCAs and metabolites (amitriptyline, nortriptyline, imipramine, desipramine, doxepin, nordoxepin, clomipramine, norclomipramine, and trimipramine) in serum that was free of interference from co-eluting compounds and other medications.
A simple method for serum was developed in which samples were precipitated by mixing serum with 50% acetonitrile and 50% methanol containing internal standards. After centrifugation the supernatant was removed and analyzed by LC-MS/MS using a quantifying and qualifying ion for each TCA or internal standard, with a total run time under 6 minutes.
A LC-MS/MS method was validated that removed known interferences that co-eluted(i.e. chlorpheniramine, diphenhydramine)in the lab's predicate HPLC assay that could contribute to falsely elevated TCA results. In addition, no interference from any of the top 25 prescribed drugs, other antidepressants and/or metabolites, or common drugs

of abuse was found. Other medications (i.e. cyclobenzaprine for the amitriptyline MRM transition; protriptyline and mephobarbital for the nortriptyline MRM transition; and nortrimipramine for the imipramine MRM transition) were also chromatographically separated after being incorrectly identifed when injected under the initial LC-MS/MS conditions. The analytical measuring range was 20-1,000 ng/mL for all compounds expect trimipramine which was 20-600 ng/mL. The intra- and inter-assay precision CVs for all compounds was <5% throughout the analytical range. Analytical accuracy was determined with a minimum of 20 samples and proficiency testing samples for each analyte. The results were compared using linear regression and all comparisons had slopes between 0.9 and 1.10, y-intercept 95% confidence intervals including 0, and $R^2 \geq 0.98$.
The timely and accurate quantification of TCA is important for both clinical and forensic applications. A highly specific and sensitive LC-MS/MS assay for serum was successfully validated and shown to be free of interferences from co-eluting compounds and other medications.

P109. ANALYSIS OF DRUGS IN SAMPLES OF ORAL FLUID FROM NIGHTCLUB PATRONS IN OSLO

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In Norway, the most commonly found illicit drugs in blood samples from criminal suspects submitted for forensic toxicological testing (including suspected drugged drivers) are THC (cannabis) and amphetamines, most often in combination with benzodiazepines. Cocaine, MDMA and other illicit drugs are rarely found. We hypothesize that the pattern of illicit drug use among nightclub patrons is different from what we observe in those forensic cases.
To investigate the use of psychoactive substances by nightclub patrons in Oslo by analysing samples of oral fluid. Research stations were established outside nine popular nightclubs with different profiles and patron-characteristics, equally distributed in the Eastern, Central and Western part of downtown Oslo. Data and sample collection was conducted on Fridays and Saturdays in March and May 2014. Individuals and groups who entered defined recruitment zones from 22:00 to 04:00 were invited to participate in this voluntary and anonymous study. Oral fluid was collected using the Intercept Oral Fluid Sampling Device. Methanol was added to increase the recovery of cannabinoids from the device. Sample preparation was performed using liquid-liquid extraction with ethyl acetate/heptane (4:1) after adding internal standards, ammonium carbonate buffer pH 9.3 and Triton X-100. The extracts were evaporated to dryness and analysed by UPLC-MS/MS using an Aquity UPLC HSS T3 1.8 µm column and positive ion mode electrospray. First, a pilot study was performed analysing 80 samples for 122 substances (psychoactive medicinal drugs, classical illicit drugs and new psychoactive substances (NPS)). Due to limited resources, the remaining samples were analysed for 46 substances, which were chosen based on findings in the 80 pilot samples and information from police and customs authorities about recent drug seizures.
The overall participation rate was 76%; 69% were below 30 years and 68% were men. Only amphetamines, cocaine and THC were found in the 80 pilot samples that were analysed for a broad range of substances. Among the total 500 samples collected during the study period, we found illicit drugs in 25.6% and medicinal drugs in 4.2% of the samples. The most prevalent substances were: cocaine/benzoylecgonine 15.0%, THC 12.4%, amphetamines 2.8%, benzodiazepines 3.2%, NPS 1.4%, MDMA 0.6% and opioids 0.3%. Combinations of illicit and medicinal drugs were found in 2.6% of the samples.
The prevalence of cocaine in the oral fluid samples was higher than in blood samples received for forensic toxicological testing, whereas the prevalence of medicinal drugs was very much lower. The results show that the drug use pattern among nightclub patrons was substantially different from the drug use pattern manifested by individuals apprehended by the police suspected for criminal conduct. The prevalence of NPS was under-estimated because only a small fraction of the substances on the market were included in the analysis.

P110. TARGETED OPIOID SCREENING ASSAY FOR PAIN MANAGEMENT USING HIGH RESOLUTION MASS SPECTROMETRY

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The use and adherence monitoring of opioids in pain management is recommended by numerous clinical practice guidelines. Many physicians use urine immunoassay screening tests, which suffer from a lack of sensitivity and specificity, to verify compliance to pain medications. However, several immunoassay tests need to be ordered to comprehensively detect the synthetic, semi-synthetic, and natural opioids due to the limited cross-reactivity of each assay. Superior testing strategies are required to specifically identify low concentrations of opioids found in adherent pain management patients.
The aim of this study was to validate a liquid chromatography tandem mass spectrometry (LC-MS/MS) method using high-resolution, accurate-mass Orbitrap detection for the qualitative identification of thirty-one opioids and metabolites (codeine, codeine-6-Beta-glucuronide, morphine, morphine-6-Beta-glucuronide, 6-acetylmorphine, hydrocodone, norhydrocodone, dihyrdocodeine, hydromorphone, hydromorphone-3-Beta-glucuronide, oxycodone, noroxycodone, oxymorphone, oxymorphone-3-Beta-glucuronide, noroxymorphone, meperidine, normeperidine, methadone, EDDP, propoxyphene, norpropoxyphene, tramadol, O-desmethyltramadol, tapentadol, tapentadol-Beta-glucuronide, N-desmethyltapentadol, buprenorphine, norbuprenorphine, norbuprenorphine-glucuronide, fentanyl, and norfentanyl).

Poster abstracts

A simple urine method was developed in which samples (100 µL) were diluted (1:10) with internal standard in clinical laboratory reagent water (CLRW) and analyzed by LC-MS/MS using high-resolution, accurate-mass Orbitrap detection with heated electrospray ionization in positive mode. A step gradient elution off of an Ultra Biphenyl (3 µM 50 x 3.0 mm) column achieved complete chromatographic separation of isobaric compounds with a total run time of approximately 8 minutes, a flow rate of 0.5 mL/min, and the ability to multiplex samples. Mobile phase A contained 10mM ammonium formate with 0.1% formic acid in CLRW, while mobile phase B contained 0.1% formic acid in acetonitrile. The following m/z ions were used to identify each of the compounds: (codeine 300.16, codeine-6-Beta-glucuronide 476.19, morphine 286.14, morphine-6-Beta-glucuronide 462.18, 6-acetylmorphine 328.15, hydrocodone 300.16, norhydrocodone 286.14, dihyrdocodeine 302.17, hydromorphone 286.14, hydromorphone-3-Beta-glucuronide 462.18, oxycodone 316.15, noroxycodone 302.14, oxymorphone 302.14, oxymorphone-3-Beta-glucuronide 478.17, noroxymorphone 288.12, meperidine 248.16, normeperidine 234.15, methadone 310.22, EDDP 278.19, propoxyphene 340.23, norpropoxyphene 308.20, tramadol 264.20, O-desmethyltramadol 250.18, tapentadol 222.19, tapentadol-Beta-glucuronide 398.22, N-desmethyltapentadol 208.17, buprenorphine 468.31, norbuprenorphine 414.26, norbuprenorphine-glucuronide 590.30, fentanyl 337.23, and norfentanyl 233.16).
A LC-MS/MS targeted screening method was validated. The cutoffs for the thirty-one opioids ranged from 2 ng/mL for fentanyl, norfentanyl, 5 ng/mL for buprenorphine, norbuprenorphine up to 25 ng/mL for most other parent compounds and non-conjugated metabolites (oxycodone, methadone, hydrocodone, hydromorphone, dihydrocodeine, norhydrocodone, etc.). The glucuronide metabolite cutoffs were set at 100 ng/mL. The intra- and inter-assay precision coefficients of variation for all compounds were <10% at concentrations 50% below the cutoff, at the cutoff, and at 50% above the cutoff concentrations for each analyte. Analytical accuracy was determined by comparing patient and proficiency testing samples for each analyte against quantitative confirmatory LC-MS/MS tests and spiked recovery experiments. The Orbitrap method showed 100% concordance with the confirmatory method for all analytes based on the individual drugs' cutoff concentrations.
The laboratory was able to successfully validate a highly specific and sensitive LC-MS/MS targeted screening assay using a high-resolution, accurate-mass method for the comprehensive detection of opioids in urine.

P111. THE IMPACT OF NEW PSYCHOACTIVE SUBSTANCE REGULATION IN JAPAN

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New Psychoactive Substances (NPS) have rapidly emerged in the market as "legal" alternatives to controlled drugs in Japan. In 2014, the Japanese government enforced the new drug control policy, which added 544 psychoactive substances as controlled drugs. The government attempted to assess the policy eight times throughout the year and decided to add generic synthetic cathinones to the listed of controlled drugs. In addition, they regularly inspected the products of NPS retailers. Each time the government changed the drug policy, retailers sought to adjust their products so as to adhere to policy but were unsuccessful. As a result, the number of NPS retailers has fallen dramatically.
The purpose of this study is 1) to address the prevalence of NPS in the Japanese market and 2) to evaluate the effectiveness of new government regulation.
Preparation of sample solution: Began with 10-50mg or 50uL of the sample, which was then extracted with 2mL of methanol under ultrasonication for 5 min. Filtration with 0.45um filter unit. Compounds were identified using GC/MS (DB5-MS Capillary column, Electrical Ionization, 70eV). The following data was used in the analysis: The Narcotics Control Department (NCD) Kanto Laboratory analyzed 1052 NPS samples in 2014. The Office of Senior Officer for NPS counted the number of NPS retailers in the nation.
The study found that the synthetic cannabinoids 5-fluoro-AB-PINACA and FUB-PB-22, and the cathinone a-PHP were the most prevalent NPS in the first half of 2014. In the latter half of the year, the synthetic cannabinoids NM2201 and 5-fluoro-ADB-PINACA, and the cathinones PV10 and 4F-octedrone were frequently found instead. Several compounds that escaped from generic scheduling of the cathinones, including indane analogs (e.g., the a-PHP indane analog: 5-BPDI) and benzofuran analogs (e.g., 5-EAPB), were also detected in late 2014. Transition of each NPS followed the legislative change. For example, 5-fluoro-AB-PINACA was found in 25.2% of the tested products before July 11, 2014 when the regulation of the substance became effective, and the ratio decreased to 6.6% after that date. Some of synthetic cannabinoids (e.g., FUB-PB-22 and NM2201) caused analytical problems due to thermal breakdown in the injection port of GC-MS. The study also found that the number of NPS retailers in the nation decreased from 230 to 13. Although at the beginning of 2014, less than 3% of NPS were found to be regulated substances, the percentage increased to over 50% by the end of 2014. One possible reason for this considerable increase is that NPS retailers may have been confused by rapid policy changes. After analyzing 1052 NPS samples, the study uncovered the most common trends and the most widely used compounds in NPS. Furthermore, as the number of controlled drugs in the new policy increased, the prevalence of NPS was successfully decreased by the government. These results indicate that enforcing strict regulation can be regarded as one of the most effective approaches.

P112. **EUROPEAN PROJECT "I-SEE" FOR STRENGTHENING INFORMATION EXCHANGE BETWEEN ITALY AND SOUTH EAST EUROPE NEIGHBORING COUNTRIES ON NEW PSYCHOACTIVE SUBSTANCES**

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The spread of New Psychoactive Substances (NPS) in Europe and worldwide is getting more and more dangerous for those who consume them. Their availability on the market, including on Internet, and their toxicity for consumers' constitute a matter of concern among health professionals, law enforcement, social operators and policy makers. There is a urgent need to identify new integrated forms of prevention, intervention and accurate monitoring of NPS at European level. One of those tools is the new European project I-SEE, granted by the European Commission, involving 5 partners: from Italy, the University of Florence, as coordinator; from Croatia, the University of Split School of Medicine; from Slovenia, the National Institute of Public Health, the Ministry of Interior Police and the Association DrogArt.
The project intends to strengthen information exchange between Italy and South East Europe neighboring countries on NPS. It supports the development and consolidation of national Early Warning System (EWS) networks, creates a joint mechanism for information exchange, mutual learning and good practice exchange among EWSs, increases information exchange towards Law Enforcement to ease and fortify activities aimed at early identifying and intercepting NPS supply.
I-SEE project is organized in 3 steps:
• Building up a network with Law Enforcement, NGOs and health sector (Slovenia). Some NGOs will be selected to collect NPS samples from drug users and transmit them anonymously to Law Enforcement to be analyzed.
• Building up clinical network (Croatia), to develop an effective network in clinical settings, including clinical toxicology laboratories, emergency wards, departments of forensic medicine and other relevant subjects in the health sector.
• Developing tools for strengthening NPS information exchange and identification in the toxicological and forensic field (Italy), by arranging a model for information flows among the three EWSs and acquiring new technical and analytical tools enabling laboratories to identify NPS.
The project aims at increasing the degree of information sharing about NPS at fours levels.
• At Law Enforcement level, the project should help each collaborating partner to strengthen planning and organizational aspects of monitoring NPS traffic and dealing.
• At analytical and toxicological level, scientific centres and laboratories participating to the project will acquire relevant knowledge and experience in identifying NPS in collected, seized and biological samples.
• At clinical level, the project aims at improving health professionals' knowledge and expertise in managing patients intoxicated by NPS through a more effective identification of signs and symptoms and the elaboration of accurate diagnosis and therapies.
• At strategic level, the resulting best practices will be shared step by step both among Italy, Slovenia and Croatia and within the national EWS.
The I-SEE project represents an added value for the whole European Union as it allows to strengthen information exchange on NPS among neighbouring countries and ease Law Enforcement activities and cooperation both within the country and among countries. The project will valorize national EWS experiences and good practice exchange and will help improving effectiveness of the European EWS for the sake of all Member States.

P113. **MASS SPECTROMETRIC ANALYSIS OF BLACK MARKET PRODUCTS WITH SUSPICIOUSLY DOPING RELEVANT INGREDIENTS BY HPLC-(HR)MS, GC-(HR)MS, ICP-MS, AND 1D-GEL ELECTROPHORESIS-UPLC-MS/MS**

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The desire for success in sporting terms, which includes not only the goals "faster, higher, stronger" by top athletes but also "more muscle and less fat" by athletes of recreational sport leads unfortunately to an extensive abuse of performance enhancing drugs. This opens a black market for original drugs and faked products from underground laboratories. The European Monitoring Center for Emerging Doping Agents (EuMoCEDA) analyzed a total of 133 products qualitatively and quantitatively in 2014. In 129 cases, 40 different doping-relevant analytes were found, from which 37% of the ingredients were not labelled.
The aim of the European Monitoring Center for Emerging Doping Agents (EuMoCEDA) is the monitoring of developments concerning doping agents´ trafficking and hence the anticipation of upcoming challenges requiring preventive actions.
Analysis was performed by high performance liquid chromatography / high resolution mass spectrometry (HPLC-HRMS) -experiments in full-scan mode. Qualification and quantification of analytes were obtained by conducting product-ion scans with substance specific fragmentation pathways. Included drugs were anabolic agents, stimulants, growth factors, natural and synthetic insulins, IGF-1 and synthetic analogs as well as growth hormone releasing factors. For gas chromatography / (high resolution) mass spectrometry (GC-(HR)MS) -experiments the

analytes were derivatised and measured in full-scan mode. Qualitative and quantitative analysis were accomplished by using reference substances and/or reference databases. Included substances were anabolic agents, stimulants, beta-2-agonists and narcotics. ICP-MS experiments were conducted for metal-analysis, including but not limited to Pb, As, and Hg. For the analysis of peptides and proteins aliquots were separated by polyacrylamide gel electrophoresis and staining. By bottom-up analysis, the identities of analytes were confirmed with nano liquid chromatography / tandem mass spectrometry. Included analytes were human growth hormone (hGh), growth factors (e.g.: FGF, MGF, etc.), various erythropoietins (EPO), and growth hormone releasing factors.
In total 56 analytes (including metals) were found, 40 agents of these have doping relevance. An amount of 46 % of all 133 analyzed products were anabolic agents, 49% were peptide hormones, growth factors, and hormones and metabolic modulators, 5% related to diuretics and stimulants. The analytes without current doping relevance were e.g. vitamins and their precursors or dermatologic agents. Remarkable findings were the discovery of heavy metals by means of ICP-MS in lyophilisates, the identification of a drug-affinity complex(DAC)-linked derivative of CJC-1295, and the analysis of the follicle-stimulating hormone (FSH) suppressing protein follistatin-344.
The statistical overview of analysed black market products shows that steroids are still popular drugs but metabolic modulators and peptidic hormones are detected with increasing frequency. Nevertheless the high amount of incorrectly labeled preparations and the content of drugs without clinical approval shows the health risk associated with drug abuse in recreational and professional sport.

P114. 5F-CUMYL-PINACA IN 'E-LIQUIDS' FOR ELECTRONIC CIGARETTES - A NEW TYPE OF SYNTHETIC CANNABINOID IN A TRENDY PRODUCT

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In recent years e-liquids used in electronic cigarettes have become an increasingly attractive alternative to smoking tobacco. Especially among young people e-cigarettes are becoming more and more popular. A new trend is the use of e-liquids containing synthetic cannabinoids instead of nicotine as active ingredients.
In the frame of the EU-Project 'Spice II Plus' comprising a systematic monitoring of the online market of 'legal highs', we bought e-liquids from online retailers who also sell herbal blends.
The products were analyzed by GC-MS using an in-house library containing mass spectra of synthetic cannabinoids. In some of the e-liquids an unidentified compound was detected. After NMR analysis it was identified as 5F-cumyl-PINACA (1-(5-fluoropentyl)-N-(2-phenylpropan-2-yl)-1H-indazole-3-carboxamide). To investigate the metabolism of this new class of compounds we incubated 5F-cumyl-PINACA and cumyl-PINACA with pooled human liver microsomes (pHLM). Cumyl-PINACA was additionally ingested orally (0.6 mg) to a volunteer in a self-experiment. To assess the relative potency of this new substance class a set of synthetic cannabinoids were characterized using the cAMP Biosensor Assay (DiscoveRx, Fremont, USA).
47 % of the e-liquids contained only nicotine as the active ingredient. The other liquids contained one or more synthetic cannabinoids, e.g. 5F-APINACA, AB-PINACA or 5F-PB-22. Three of the liquids sold as 'c-liquids' (all from one retailer) contained 5F-cumyl-PINACA. Metabolism of 5F-cumyl-PINACA and cumyl-PINACA was similar to the metabolism of AM-2201 and JWH-018. The main metabolites show hydroxylation at the pentyl moiety. The main metabolites detected in the volunteer's urine sample were the same as in the pHLM assay. The volunteer did not experience any drug-related symptoms after ingestion of cumyl-PINACA. All synthetic cannabinoids tested with the cAMP assay were full agonists at the CB1-Receptor. Cumyl-PINACA was the most potent synthetic cannabinoid among the tested compounds and showed an EC50 of 0.06 nM (JWH-018: 1.13 nM).
The increasing popularity of e-liquids particularly among young people and the extreme potency of the added synthetic cannabinoids pose a serious threat to public health. There is a high risk of unintended poisoning, and in the long-term prevalence of these drugs could rise in the younger population due to introduction of trendy products.

P115. STABILITY OF PROPOFOL IN SAMPLED ANTE-MORTEM BLOOD

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Propofol is an intravenous anaesthetic agent used for the induction and maintenance of anaesthesia. Propofol is also used recreationally due to its sedative and relaxing properties and has been involved in fatalities. Thus, for several reasons, an exact determination of the propofol concentration in clinical and forensic samples may be required. Due to the time lag between sampling and analysis, which may account for several days, it is essential to be aware of the stability properties of propofol.
The aim of the present study was to provide knowledge about the stability of propofol and propofol glucuronide in collected ante-mortem blood samples stored under different temperature conditions and with the use of different additives. The stability was investigated using both spiked whole blood samples and clinical samples with an authentic content of propofol and propofol glucuronide. All of the samples were stored in two different blood collection tubes: Venosafe VF-054SFX tubes (Terumo Europe) containing 9 mg of sodium fluoride (NaF) and 9 mg of potassium oxalate (FO mixture) for a 4-mL draw volume of blood and Venosafe VF-053SFC32 tubes containing 6.8 mg of NaF and 15.7 mg of citrate-EDTA buffer ingredients (FC mixture) for a 3-mL draw volume of blood. The spiked samples were prepared at concentrations of 0.05-3 mg/L of propofol and twice that of propofol glucuronide. The solvents of the spiked solutions were evaporated before mixing with the blood. The samples were stored at ambient temperature, 5°C, -20°C and -80°C for up to 4 months. Analyses were performed periodically during the storage using a sensitive

LC-MS/MS method that simultaneously determines the two analytes.
Samples spiked with propofol to a concentration of 0.05-3 mg/L showed a 20-40% reduction in concentration over a 2-week period when stored at -20°C in FC tubes. The concentration of propofol also declined in the FO tubes but at a pronounced lower rate. Instability was not observed at 5°C or -80°C, even after prolonged storage. No significant instability of propofol glucuronide was observed during a 4-month storage period, irrespective of the storage conditions. The observed instability of propofol at -20°C was verified using clinical samples with authentic propofol contents. The samples were collected in both FC and FO tubes and stored at -20°C. In a representative case, the analysed propofol concentrations were 0.96, 0.29, 0.24, 0.14 and 0.07 mg/L in FC tubes and 0.98, 0.97, 0.89, 0.74 and 0.33 mg/L in FO tubes after 6, 18, 43, 74 and 330 days of storage. The atypical stability pattern of propofol may be related to its antioxidative properties. At -20°C the reductases in blood may not efficiently prevent oxidative changes caused by for example autoxidation. The lesser instability of propofol in the FO-tubes may be due to the reducing properties of oxalate.
Propofol is instable in whole blood mixed with FC and FO additives when stored at -20°C. The rate of concentration decline was greatest when the FC additive was used. The stability was improved considerably by storage at 5°C and -80°C.

P116. INTRODUCTION OF SAMPLE TUBES WITH SODIUM AZIDE AS A PRESERVATIVE FOR ETHYL GLUCURONIDE IN URINE

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Bacterial degradation of ethyl glucuronide (EtG) and neo-formation of EtG from ethanol in samples containing bacteria have been reported. World-wide, no preservatives for stabilizing the analyte concentrations are used, although shipping of samples may take several days under non-cooled conditions. We have systematically investigated preservatives for microbiological degradation, especially the use of boric acid tubes has been recommended. However, these have not been introduced in routine, since boric acid is not compatible with EtG immunoassay. Sodium azide is a well-established preservative against microorganisms. It is known to generally inhibit microbial activity and subsequently bacterial growth. Hence it is usually used as positive control in different approaches to measure different microbial activities such as biodegradation (OECD tests).
To introduce sodium azide in EtG urine testing - as a preservative against bacterial degradation of EtG during shipping and storage of samples.
Sodium azide is present in commercially available tubes for oral fluid sampling (Greiner-Bio-One GmbH). They can be used in combination with the respective urine sample cups. Vacuum is used for suction of the urine from the sampling cup. Usually, sample volumes of 2.8 to 3.5 mL are used, with 4 mg sodium azide per tube. Urine samples spiked with EtG in the concentration range of 250 to 1250 ng/mL were then analysed by immunoassay (DRI) and in parallel by LC-MS/MS (fully validated method), using negative ESI with a Sciex API 3200 QTrap in MRM-mode, a 150x2mm, 4 µm, synergi polar-RP column (Phenomenex), and gradient elution with 0.1% formic acid (solvent A) and 0.1% formic acid in acetonitrile (solvent B) (2 to 90% solvent B in 3 minutes, 0.3 mL/min flow rate), using D5-EtG (and D5-EtS) as internal standards.
Method comparison showed good correlation of results obtained from both methods, with inaccuracy of less than 5 % for LC-MS/MS and less than 20 % for immunoassay. No matrix effects were detected resulting from the addition of sodium azide, when comparing urine samples with sodium azide to urine samples without sodium azide (collected in a plain tube). Preliminary results show, that in 36% of clinical urine samples (out of 46) EtG degradation has been found by E. coli which could be avoided by the addition of sodium azide.
The pre-analytical stability is an important issue in urine testing for ethyl glucuronide. We strongly recommend sodium azide as a preservative for EtG, thus making sure, that bacterial growth is hindered and microbiological degradation of EtG and post-sampling formation of EtG by bacteria are avoided.

P117. TREND OF MITRAGYNINE IN MALAYSIA

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The use of kratom leaves (locally known as 'ketum') is prohibited in Malaysia under Poisons Act 1952. Certain parties have urged the government to penalize the use of kratom under the Dangerous Drugs Act instead of the Poisons Act which will carry heavier penalties, not only to the kratom's dealers but also to the user. All possession cases of kratom leaf (mitragynine) and its extracted liquid have been taken to action. However, many cases of mitragynine abuse in urine were dropped because mitragynine can not be traced especially in a lower concentration. An established method has not been developed yet in Malaysia's Laboratory.
In order to performed analysis of Mitragynine in urine, Department of Chemistry Malaysia tries to develop an established method of Detection of Mitragynine in urine by GCMS. A study was carried out to trace mitagynine in a lower concentration.
A simple sample preparation consisted of a roller mix with phosphate buffer pH 12 and chlorobutane for 1 hour and followed by liquid/liquid extraction with Ethanol and hexane. The upper layer was discarded and the lower layer was dried. Recon with 80ul of Ethanol (99.98%) and analysed using GCMS.
From the previous cases, MS chromatogram shows that mitragynine can be detected especially in a higher concentration. Mitragynine was detected in urine using a simple method Liquid-Liquid Extraction (LLE) and GCMS routine analysis. However, a further studies need to be done to identify the limit of detection of mitragynine in urine.

Poster abstracts

P118. **THE INTEREST OF COLLABORATION BETWEEN TOXICOLOGIST AND CLINICIAN DEALING WITH A MERCURY EXPOSURE CASES: A CASE STUDY**

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In clinical toxicology, clinical findings do not always provide a confirmation of a diagnosis of poisoning; further investigations might be necessary to specify the nature of the intoxication or to eliminate a toxic process.
This study aims to illustrate how a useful collaboration between clinician and toxicologist could optimize the diagnostic and therapeutic orientations when dealing with acute elemental mercury exposure.
The clinical case is reported according to the medical records of a patient admitted to the medical neurology department of the University-Hospital of Setif.
A 55-year-old man with a history of a deep stomachache and constipation ingested elemental mercury in a single take to relieve his gastralgia. Four days later, he was hospitalized for impairment in motor function of the lower extremities. The abdominal X-rays showed evidence of mercury beads deposited in the intestine fields and the clinician believed that the clinical situation is associated to mercury poisoning which involved the administration of chelating agents. However, during admission, there was no kidney dysfunction, no neuropsychiatric symptoms or obvious gastrointestinal and hepatic complications. The toxicologist argued that there is no significant correlation between mercury toxicity and the clinical data and he suggested an enema to relieve constipation and to clear mercury from the gut because elemental mercury is poorly absorbed by ingestion (less than 0.01%) and its prolonged retention may facilitate its conversion to divalent mercury and its subsequent absorption. In these circumstances a mercury analysis was not required and after elimination of a possible lead poisoning (blood lead level less than 1 µg/dl), another investigative leads were explored and the findings revealed adenoma with lumbar spinal cord compression explaining the patient's motor deficit; it was a bone metastases.
This case study leads to reviewing the main knowledge of the elemental mercury toxicity that clinicians should know. Diagnosis of elemental mercury poisoning involves determining the route of exposure in first place. The collaboration between clinician and toxicologist could help to confirm or invalidate a diagnosis.

P119. **DIRECT DRUG TESTING IN ORAL FLUID BY TOUCH SPRAY-MASS SPECTROMETRY WITH MEDICAL SWABS**

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Swab touch spray (STS) mass spectrometry (MS) is a spray-based ambient ionization technique in which a medical swab is used to collect the sample, and ionization is performed subsequently, directly from the porous swab via electrospray-like mechanisms. This new adaptation of touch spray, originally using metallic teasing probes for surgical applications, allows for direct, non-invasive and in vivo biofluid analysis, e.g. oral fluid. The use of commercial medical swabs for both sample collection and ion generation has potential for rapid point-of-care testing in clinical applications, as well as in situ roadside drug testing.
We present a STS study for oral fluid testing to detect common illicit drugs. This one-step STS-MS approach is intended as an alternative analytical protocol to a dual-step procedure involving screening by immunoassays followed by confirmatory laboratory tests.
Oral fluid specimens were spiked with a mixture of 14 target drugs and 5 deuterated internal standards, or analyzed unadulterated as blanks. Rayon straight swabs with aluminum wire handle and rayon tip (Copan Diagnostics, IT) were dipped in oral fluid to absorb the specimen (about 40 µL), dried for a short period, and then held in front of the MS inlet vertically to respect to the ground. Acetonitrile with formic acid was applied to the swab tip in rayon via a fused silica capillary at a steady rate; high voltage (+6 kV) was applied to the metallic swab handle via the instrument's high voltage cable and a copper clip. Data acquisition was started concurrently with high voltage application and formation of a Taylor cone, indicating the onset of ion production. Drugs were detected via sequential product scans in MS_3 using a linear ion trap benchtop mass spectrometer (Thermo Scientific, CA). MS_3 scans proved to provide adequate specificity in the absence of chromatographic separation. Analysis time is rapid (<1 min).
Fourteen drugs, including cocaine, methamphetamines and opiates, were reproducibly identified at ng/mL levels by multiple stages of mass analysis, meeting cut-offs sought by international guidelines (LOD values ranged from 1 to 50 ng/mL); exceptions being buprenorphine and $\Delta 9$-THC that require on-line derivatization to increase the ionization efficiency. Semi-quantitation is feasible, using freshly prepared calibrators, but absolute quantitation has not been pursued. Alternative scan types to reaction monitoring are currently investigated to develop MS-based untargeted drug testing methods. Effects of swab shape, orientation, and distance from the inlet on the formation of the spray plume are currently under study, as well as the optimal properties of the solvent system to generate stable electrospray and efficient ionization, and inherent matrix effects.
Proof-of-concept results indicate that the STS-MS detection is sensitive and specific to identify several drugs in the ng/mL range. The adoption of an existing technology (i.e. swabs) expedites the translation of this novel methodology to the application pursued. However, current performance requires extension to develop a multiplexed assay, and refinement in the analytical procedures to meet clinical and legal requirements.

P120. TOXICOLOGICAL ANALYSIS IS A USEFUL TOOL TO DETECT NON-ADHERENCE TO THERAPY WITH ANTIHYPERTENSIVE DRUGS PRIOR TO RENAL DENERVATION

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Hypertension is a common medical disorder which is treated with antihypertensive drugs, typically with a multi-drug regimen. In severe cases this is not sufficient and such cases with apparent treatment resistant hypertension (TRH) are elected for catheter-based renal denervation (RDN). Most clinics have no means to assess and exclude non-adherence to antihypertensive medication as possible cause of TRH. In a previous study 85 % of patients with TRH took less than half of their medication. This highlights that non-adherence rates are cohort specific.
The Aim of the present study was to create a simple, sensitive and fast analytical method for detection of antihypertensive drugs in urine and to find out the adherence / non-adherence rate of patients that underwent RDN.
Urine samples of 79 patients with true TRH were retrospectically analyzed for antihypertensive drugs. The patients have been included in two studies that focused on potential antihypertensive and nephroprotective effects of RDN. The urine samples had been collected prior to renal denervation. The analytical procedure consisted of liquid-liquid extraction of 0.2 ml of urine with 1 ml of ethyl acetate and LC-MSMS analysis of the evaporated organic phase reconstituted in 100 µl of mobile phase. In 4 min run time (7.2 min sample to sample) 45 antihypertensive drugs and metabolites could be detected, 3 of these in negative mode (hydrochlorothiazide, furosemide and xipamide).
The present method was a progress to a previous LC-TOF MS method. The present method provided higher sensitivity and it was possible to detect low concentrations of lercanidpine which is excreted almost completely in form of metabolites. The 79 patients, which underwent RDN, were prescribed in median 6 antihypertensive drugs (range 3 - 9). Using urine analysis complete adherence was detected in 44 (56%), one drug was missing in 22 (28 %), two or more in 13 patients (16 %) of whom 3 took no medication at all (4 %).
Non-adherence to antihypertensive medication is thought to be one major cause of TRH, which is almost impossible to detect in clinical practice. The present study shows that a toxicological analysis of one urine sample can detect adherence and non-adherence, respectively. Interventional strategies in non-adherence patients and/or intensification of drug therapy can be tailored accordingly. This is advantageous not only in medical views but also in view of cost effectiveness.

P121. SIMULTANEOUS DETERMINATION OF BUPROPION, CITALOPRAM, FLUVOXAMINE AND SERTRALINE IN HUMAN SERUM BY SPE-GC/MS FOR THERAPEUTIC DRUG MONITORING PURPOSES

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A simple analytical methodology for monitoring the therapeutic serum concentrations of the antidepressants bupropion, citalopram, fluvoxamine and sertraline in psychiatric patients was applied and validated. It was developed in the context of a research program on the Therapeutic Drug Monitoring (TDM) of psychiatric drugs, in the region of Epirus, Greece, that was funded by the National Strategic Reference Framework (NSRF) 2007-2013 of the European Community.
The monitoring of therapeutic and sub-therapeutic serum levels of bupropion, citalopram, fluvoxamine and sertraline with a simple SPE-GC-MS methodology suitable for routine application.
An aliquot of 1.0 mL serum was spiked with 10 µL internal standard (IS) solution to a final concentration 100 ng/mL (internal standard was cyclobenzaprine and was used for the quantitative determination of all analytes), then was mixed with 3.0 mL phosphate buffer (pH 9.5, 0.1 M), vortexed and centrifuged. The supernatant was loaded to a Chem Elut SPE cartridge. Elution was performed twice with 5 mL of a freshly prepared mixture of dichloromethane: isopropanol (90:10, v/v). Extracts were evaporated to dryness in a stream of nitrogen, redisolved with 50 µL ethyl acetate, and an aliquot of 1.0 µL was injected onto the GC-MS. Separation of analytes were achieved on an Equity 5 capillary column (30 m × 0.25 i.d. mm, 0.25 µm). Oven temperature was programmed from 120°C held for 2 min, ramped at 20°C/min to 290°C with a final hold time 5 min (total acquisition program time: 15.50 min). The carrier gas was helium at a flow rate of 0.8 mL/min. Injector and interface temperatures were 260° and 300°C, respectively. The mass spectrometer was operated at electron impact ionization mode (EI, 70 eV). Three ions per analyte were used for acquisition in the selected-ion monitoring (SIM) mode. The basic ion of each drug was used for the quantification of each analyte. Quality criteria were as follows: retention times of analytes did not differ more than±0.1 min from the expected time, and the relative intensities of the selected ions did not differ more than 10% from the relative intensities of the same ions acquired from a standard sample for each analyte.
Absolute recoveries were found to be higher than 82% for all analytes. The linearity was good with a correlation coefficient (R^2) ranged from 0.9857 to 0.9971. The intra-day repeatability ranged from 2.69% to 9.38% and the inter-day reproducibility ranged from 4.31% to 11.65%. LOQs ranged from 10 to 60 ng/mL. The method was applied successfully for monitoring the concentrations of these drugs in psychiatric patients.
The present work describes a simple SPE-GC-MS method for the simultaneous determination of bupropion, citalopram, fluvoxamine and sertraline in human serum.The proposed analytical methodology has analytical features which allow its application for the TDM of these four antidepressants: simple SPE and direct GC-MS analysis, without derivatisation step; short analysis time; determination of toxic, therapeutic and sub-therapeutic serum concentrations.

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P122. GENOTOXIC DAMAGE AND OCCUPATIONAL EXPOSURE TO FORMALDEHYDE IN ANATOMIC PATHOLOGY LABORATORY WORKERS

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Formaldehyde is a genotoxic, mutagenic and carcinogenic chemical routinely used in anatomic pathology laboratories. Levels reported in workplace air often exceed permissible exposure limits and preventive measures are insufficient; which raises a problem in occupational health.
To evaluate genotoxic damage and occupational exposure to formaldehyde in workers of anatomic pathology laboratories.
The study was carried out in 42 health workers exposed to formaldehyde from three anatomic pathology laboratories and 38 unexposed workers. Exposure level to formaldehyde in the workplace air was evaluated by applying spectrophotometric method with chromotropic acid. Evaluation of genetic damage was performed by applying genotoxicity biomarkers such as micronucleus and nuclear abnormalities frequency in epithelial cells from the buccal mucosa and comet assay in capillary blood lymphocytes.
The mean concentration of formaldehyde in air was 0.96 mg/m3 (min = 0.311 mg/m3, max = 1.466 mg/m3), exceeding the threshold limit value (TLV-ceiling = 0.37 mg/m3). Workers exposed to formaldehyde had micronucleus, nuclear buds and binucleated cells frequencies significantly increased in relation to the unexposed group ($p<0.01$). On the other hand, no significant differences were found in any of the comet assay parameters between study groups ($p>0.05$).
Workers in anatomic pathology laboratories showed exposure to high concentrations of formaldehyde in the workplace and had increased genotoxic damage in the buccal mucosa. These results, coupled with carcinogenic activity and insufficient measures to prevent exposure, point out a high occupational risk situation, which must be addressed by government agency responsible for occupational health and safety by implementing a comprehensive risk management program.

P123. DEVELOPMENT OF AN LC-MS/MS METHOD FOR THE DETERMINATION OF ENDOGENOUS CORTISOL IN HAIR USING 13C-LABELED CORTISOL

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Hair cortisol levels are applied increasingly as a measure for stress in humans and mammals. Cortisol is an endogenous compound and is always present in hair. Therefore, "cortisol-free hair matrix" is a crucial point for any analytical method to accurately quantify low cortisol levels.
The aim of this project was to modify current methods used for hair cortisol analysis to more accurately determine low endogenous hair cortisol concentrations. Therefore, the use of $^{13}C_3$-labeled cortisol instead of unlabeled cortisol for production of the calibrators of a validation was assessed. The $^{13}C_3$-labeled cortisol should have identical physicochemical properties compared to the unlabeled cortisol but is typically not present in hair. A comparative validation applying cortisol versus $^{13}C_3$-labeled cortisol should finally document the performance of this concept.
Cortisol was extracted from 20 mg hair (standard sample amount) applying an optimized single step extraction protocol. An LC-MS/MS method was developed for the quantitative analysis of cortisol, $^{13}C_3$- and D4-cortisol, and D7-cortisone as internal standard (IS). All these compounds were detected as their formic acid adducts in negative ESI on a Qtrap 5500 (ABSciex). For validation the following parameters were tested using either cortisol or $^{13}C_3$-labeled cortisol: Selectivity, Limit of detection (LOD), limit of quantification (LOQ), linearity, matrix effect, recovery, accuracy and precision. Endogenous cortisol concentrations were also determined by standard addition.
An LC-MS/MS method was established for all compounds. Cortisol and $^{13}C_3$-labeled cortisol eluted at the same retention time (5.74 min). Because of interferences with $^{13}C_3$-cortisol, D4-cortisol had to be replaced by D7-cortisone as internal standard. No interferences with cortisol were observed for $^{13}C_3$-cortisol. The two methods (cortisol / $^{13}C_3$-labeled cortisol) were validated in a concentration range up to 500 pg/mg. As expected, differences of some validation parameters were observed for LOD (0.2/0.1) and LOQ (1/0.5) and QC low (2.5 pg/mg) which showed Matrix effects (126.5/79.3 ,SD 35.5/6.6) when using cortisol to spike. The matrix effect can be explained by the unknown amount of endogenous cortisol that is present in the different hair samples used to determine matrix effects. No matrix effects were observed for the high QC (400 pg(mg) samples. Recovery was 92.7/87.3 (SD 9.9/6.2) for QC low (2.5 pg/mg) and 102.3/82.1 (SD 5.8/11.4) for QC high (400 pg/mg). The variable endogenous cortisol concentration of unknown samples was also determined by a standard addition experiment and was in good agreement with results calculated from the $^{13}C_3$-labeled cortisol calibration curve. The $^{13}C_3$-labeled cortisol method was finally applied to determine cortisol levels in humans and mammals.
An LC-MS/MS method for determination of cortisol in hair was successfully established and validated. It could be shown that the use of $^{13}C_3$-labeled cortisol for calibration was advantageous for accurate quantification of low endogenous cortisol levels.

P124. QUANTIFICATION OF DRUGS FOR DRUG-FACILITATED CRIMES IN HUMAN URINE BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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A considerable increase in the number of reported drug-facilitated crimes (DFC) has occurred in recent years. Drugs that can induce a state of semi- or unconsciousness in the victims are most typically used in these cases; victims are usually unable to fight off their attackers and report their inability to prevent the crime as it occurs. Sedative-hypnotic drugs like norketamine, phencyclidine (PCP) and γ-hydroxybutyric acid (GHB) are among the compounds most frequently involved in this kind of offences.
To implement a liquid chromatography tandem mass spectrometry analytical method for the quantification of drugs for drug-facilitated crimes in human urine on a Thermo Scientific™ TSQ Quantum Access MAX™ triple quadrupole. A calibration curve covering the concentration range 5-200 ng/mL was prepared in duplicate by spiking blank human urine with methanolic solutions (50x) containing ketamine and its metabolites norketamine and dehydronorketamine and PCP. 300 µL of a 100 ng/mL solution containing ketamine-D4 and PCP-D5 in methanol with 0.1% formic acid were added to 100 µL of each calibrator; a blank urine sample was also added. Due to the high concentrations involved, a calibration curve of GBL in methanol was prepared to cover the concentration range of 1-100 µg/mL; this range was based on the assumption of an endogenous level for GHB in urine of 10-50 µg/mL. 100 µL of each calibrator, including a blank, were added to 300 µL of a 50 µg/mL solution of GHB-D6 and GBL-D6 in water/methanol/formic acid 33/67/0.1 (v/vv). All samples from the two extracted calibration curves were vortex-mixed, centrifuged and the supernatant injected onto an LC-MS/MS system. Chromatographic separation was achieved on a Themo Scientific™ Hypersil GOLD™ column (150 x 2.1 mm, 3 µm) at room temperature using gradient elution with a mobile phase consisting of water and methanol both containing 0.1 % formic acid. Detection was performed by single reaction monitoring (SRM) on a TSQ Quantum Access MAX triple quadrupole mass spectrometer using heated electrospray ionization in positive mode.
The assay proved to be linear for all the analytes of interest in the calibration range specified above using a linear interpolation with 1/x weighing. The percentage bias between nominal and back-calculated concentration for the calibrators was between -10.6% and 12.3%; the correlation factor (R^2) was always above 0.998. The method also proved to be able to detect GHB in human urine above the endogenous level; a signal-to-noise ratio above 400 was obtained for GHB in the three human urine samples analysed.
A liquid chromatography tandem mass spectrometry method for the quantification of a panel of drugs for DFC and their metabolites in human urine has been developed on a TSQ Quantum Access MAX. The instrument proved to have the sensitivity and linearity of response suitable to cover the necessary range of concentration for these drugs.

P125. THERAPEUTIC MONITORING OF ANTIEPILEPTIC DRUGS

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Therapeutic monitoring currently represents a compelling alternative to rationalize therapeutic methods. The routinization of therapeutic monitoring of pharmacologically active substances have a narrow therapeutic window is indispensable.
This study aims to optimize the management and monitoring of patients treated with antiepileptic drugs, in order to ensure better safety and efficacy of treatments.
It is a multicenter non-interventional study in two departments: neurology and pediatrics, in university-hospital of Tlemcen. This prospective study was conducted on a random sample of 32 epileptic patients with a sex ratio of 15/17 treated with at least Valproic acid and / or carbamazepine. Plasma assay was carried out by enzymatic method in the homogeneous phase using a AxSYM analyzer. Among the 32 patients included in the study, 29 patients were treated with valproic acid, of whom 20 patients with mono-therapy and 6 are stabilized by a combination therapy. 3 patients received a polymedication. For 47% of patients the duration of treatment was between 1 and 5 years. 15 patients were declared to be not stabilized by treatment, of whom 3 received a treatment combining three antiepileptic dugs.
34.5% of patients treated with valproic acid have plasma concentrations less than 50 mg / l while 65.5% of patients have plasma concentrations included in the therapeutic range. No patient has a higher plasma concentration 100mg / l. For 50% of patients treated with carbamazepine in bi or combination therapy, 33% had therapeutic plasma concentrations. It is noted that 34.40% of the patients were under dosed. The study of the correlation between plasma levels of antiepileptic drugs and stability of epilepsy showed four different situations; patients with normal doses and stable condition (44%), patients with normal doses unstable condition (22%), underdosing patients with unstable condition (25%), underdosing patients with stable condition (9%). This study shows a debatable inefficiency of the combination of two antiepileptic drugs and a total ineffectiveness of combination of three antiepileptic drugs.
It seems imperative to take effective measures to improve the management of patients with epilepsy. The existence of a unit of therapeutic drug monitoring of proximity that works on a regular basis in order to optimize treatment strategies and reduce the risk of drug interactions. Secondly, a collaborative clinical effective and efficient biological.

Poster abstracts

P126. **VALIDATED LC-MS/MS METHOD FOR QUALITATIVE AND QUANTITATIVE ANALYSIS OF 75 SYNTHETIC CANNABINOIDS IN SERUM**

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The market of synthetic cannabinoids is changing very fast and therefore it is necessary to provide methods for the identification and quantification of synthetic cannabinoids in human serum. For use in forensic casework these methods need to be adapted to the range of available substances and should therefore allow for easy updating and revalidation.
The aim of this work was to have a fully validated LC-MS/MS method, which can be used in forensic cases for the quantitative and qualitative analysis of synthetic cannabinoids in serum.
Analysis is performed by using a liquid-liquid-extraction applying two different extracting agents (1 ml serum; 1.5 ml hexane/ethylacetate (99/1, v/v) and 1.5 ml hexane/ethylacetate (80/20, v/v), respectively) and an LC-MS/MS system (QTrap 4000, ABSciex, equipped with a Kinetex C18 100 Å (100 x 2,1 mm, 2,6 µm) column, Phenomenex, Injection volume: 20 µl). Validation was carried out in accordance with the guidelines of the GTFCh [Guidelines for quality assurance in forensic-toxicological analyses, Appendix B - Requirements for the validation of analytical methods, Toxichem Krimtech (2009) 76 (3): 185-208].
Overall selectivity and specifity was sufficient for all analytes. 59 of the substances met the requirements for linearity and accuracy and can therefore be accurately quantified with limits of quantification (LOQ's) ranging from 0.1 to 2.0 ng/ml. 14 of the substances can be analyzed semiquantitatively, because accuracy was outside the acceptable range of ±20 % (but lower than ±30 %). Two of the substances (XLR-12 and ADB-PINACA-5F) can only be analyzed qualitatively because accuracy and linearity were not sufficient. The LOQ's were set to the lowest calibration point (0.1 ng/ml) for most of the compounds. 15 of the analytes showed a higher LOQ. After adding new compounds to the method, a revalidation is carried out including at least selectivity, specificity, linearity, limits of detection and matrix effects.
The method was validated for 75 compounds. 59 can be quantified precisely, 14 are determined semiquantitatively and two qualitatively. The group of compounds carrying a valine amide (or structurally related) moiety showed relatively strong matrix effects and therefore yielded higher LODs and LOQs. To compensate for matrix effects, the use of a deuterated internal standard is advised, and for some of these analytes deuterated analogues are available now. Since the validation was completed, 35 new substances were added to the method (as of March 2015) and a revalidation will be carried out soon.

P127. **COMPARISON BETWEEN URINE, BLOOD, SALIVA, AND FINGERPRINTS IN FORENSIC DRUG TESTING**

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It is important to take biological specimens as soon as possible to prove drug intake because drugs in the body are gradually metabolized and excreted. However, restrictions such as trial procedures and the security of staff and facilities delay the immediate sampling of urine and blood. Saliva and fingerprints (secretions from fingertips) are spontaneously and non-invasively secreted and can be sampled quickly and easily on site by police officers. Sampling saliva or fingerprints places a smaller physical and mental burden on suspects than sampling urine or blood. We evaluated the effectiveness of saliva and fingerprints as alternative specimens to urine and blood in terms of ease of sampling, drug detection sensitivity, and drug detection periods for each specimen type.
After four commercially available pharmaceutical products were administered, each in a single dose, to healthy subjects, their urine, blood, saliva, and fingerprints were taken at predetermined sampling times over approximately four weeks. Their urine was excreted into a plastic cup, and their saliva, which was spontaneously secreted in their mouths, was spat out onto a plastic dish after rinsing their mouths with water. Their blood was drawn from a finger by pricking any of the medial or lateral surfaces of fingers with a lancet. To sample fingerprints, they pressed each of their index fingertips for 30 s onto an individual filter paper that had been previously wetted with distilled water in a plastic dish after thoroughly washing with hand soap and tap water to remove any external surface contaminants. Fourteen analytes (the administered drugs and their main metabolites) were extracted from each specimen using simple pretreatments, such as dilution and deproteinization, and were analyzed using a liquid chromatograph-tandem mass spectrometer.
Most of the analytes were detected in saliva and fingerprints, as well as in urine and blood. The time-courses of drug concentrations were similar between urine and fingerprints, and between blood and saliva. Compared to the other compounds, the acidic compounds, e.g., ibuprofen, acetylsalicylic acid, etc., were more difficult to detect in all specimens. Acetaminophen, dihydrocodeine, and methylephedrine were detected in fingerprints at later sampling times than in urine. However, a relationship between the drug structures and their detection periods in each specimen was not found.
Saliva and fingerprints could be easily sampled on site without using special techniques or facilities. In addition, fingerprints could be immediately analyzed after simple and rapid treatment. In cases where it would be difficult to immediately obtain urine and blood, saliva and fingerprints could be effective alternative specimens for drug testing.

P128. IMMUNOASSAY SCREENING IN URINE FOR SYNTHETIC CANNABINOIDS – A FEASIBLE APPROACH FOR FORENSIC APPLICATIONS?

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In therapeutic settings requiring abstinence, reliable screening methods for drugs of abuse and especially for new psychoactive substances like synthetic cannabinoids are needed. For economic reasons institutions of drug rehabilitation and forensic psychiatric hospitals started to use immunoassays to screen urine samples for synthetic cannabinoids. However, the wide structural diversity of this drug class makes it difficult to design suitable antibodies, and wrong negative results can impede the therapeutic process.
A retrospective study was performed to check if two commercially available immunoassay kits are capable of detecting currently prevalent substances in authentic urine samples.
Urine samples of over 500 individuals from seven different forensic psychiatric hospitals located in the federal states of Bavaria and Baden-Württemberg were analyzed for synthetic cannabinoids using two commercially available immunoassays (a 'JWH-018' kit as well as an 'UR-144/XRL-11' kit). All samples were also analyzed by an up-to-date LC-ESI-MS/MS screening method covering main metabolites of 43 synthetic cannabinoids.
None of the patients was tested positive by either of the two immunoassays. In contrast, using LC-MS/MS analysis metabolites of synthetic cannabinoids were detected in 7.7 % of the samples. Detected substances were metabolites of AB-CHMINACA, AB-FUBINACA, AB-PINACA, APICA, JWH-122 and PB-22-5F. There were no marked differences regarding the positive rates across the two federal states or between hospitals applying immunoassay screening versus other means of abstinence control.
The results can be explained by insufficient cross reactivity of the available antibodies for the 'new generation' synthetic cannabinoids. Another factor could be the generally low analyte concentrations in urine and a lower sensitivity of the IA tests. In the light of this very heterogenic group of substances the use of immunoassays has to be seen critically. Particularly in the forensic field it is strongly recommended not to rely on the evaluated IA tests for synthetic cannabinoids. As the antibodies used for immunoassays of other providers probably show similar cross reactivity, similar results can be expected for other commercially available immunoassay products.

P129. METABOLITES OF SYNTHETIC CANNABINOIDS IN HAIR – PROOF OF CONSUMPTION OR FALSE FRIENDS FOR INTERPRETATION?

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Hair testing can be a useful tool for retrospective elucidation of drug use, but the issue of external contamination can hamper interpretation. Generally, the detection of drug metabolites in hair is considered as a proof for consumption. For synthetic cannabinoids, which comprise compounds carrying labile ester or amide bonds, not much is known about mechanisms of incorporation into hair. For a correct interpretation regarding findings of these compounds and their metabolites/cleavage products it is necessary to be aware of the different potential routes of incorporation.
An authentic hair sample of a patient with a well-known history of heavy consumption of synthetic cannabinoids was analyzed in segments. To enable a valid interpretation of the results regarding the distribution of analytes and their metabolites along the hair shaft, stability of 5F-PB-22 and AB-CHMINACA was tested under smoking conditions and during storage of the hair sample.
The authentic hair sample was cut into ten segments and screened for synthetic cannabinoids and their metabolites with two different LC-ESI-MS/MS methods. In addition one test person rubbed AB-CHMINACA and 5F-PB-22 into his head hair to simulate external contamination. One week later head hair samples were taken and homogenized. One part of this hair sample was washed with solvents, the other part not. Each part was divided into four fractions. One fraction was directly analyzed using the same methods and the other fractions were stored under different conditions (darkness at room temperature; daylight at room temperature; darkness at 50°C) for one week and then analyzed. Joints containing 10 mg AB-CHMINACA or 10 mg 5F-PB-22, respectively, were burned down via a water jet pump and the smoke sucked through a wash bottle filled with methanol. The condensates were screened with GC-MS. Identity of the detected substances was verified by LC-MS/MS (enhanced product ion scan).
In the authentic hair sample 5F-PB-22 and AB-CHMINCA as well as their metabolites 5F-PB-22-3-carboxyindole, PB-22-5-OH-pentyl and AB-CHMINACA valine metabolite were detected in all segments. Furthermore, trace amounts of 5F-AMB, AB-FUBINACA, AB-PINACA, BB-22, EAM-2201, NNEI and THJ-2201 were detected in some segments. 5F-PB-22-3-carboxyindole and AB-CHMINACA valine metabolites were also detected in the externally contaminated, stored samples. Highest signals were received after storage at 50 °C. The same compounds were also found in the smoke condensates besides further pyrolysis products.
Comparing the results of the authentic hair sample with the anamnestic data and the time of availability of 5F-PB-22 and AB-CHMINACA on the European market, it is evident that findings in the hair segments do not correlate with drug use in the time period the respective segments have grown. The findings could be explained by pyrolytic 'metabolite' formation during smoking and condensation on the hair or formation of these compounds over time after deposition in the hair shaft. Additionally, incorporation via sebum could have contributed to the distribution. Therefore, interpretation of 'metabolite' findings of chemically labile compounds should be carried out with utmost care, taking into account the different mechanisms of formation and incorporation into hair.

Poster abstracts

P130. HAIR TESTING IN CLINICAL SETTING: MONITORING OF 91 XENOBIOTICS IN 300 MIGRAINE PATIENTS BY LC TANDEM MS

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Migraine patients with recurrent attacks are a population at risk of overuse and abuse of analgesic medication. Triptans, selective agonists at 5-hydroxytryptamine 1B/1D (5-HR1B-1D) receptor subtype, are recommended as first-line drugs for the treatment of migraine attack (acute treatment) in patients suffering from moderate-severe migraine. However, these patients took other medications such as opioids analgesics for the attacks treatment, antidepressants and antiepileptics for prophylaxis treatment and benzodiazepines, non-benzodiazepines hypnotics (z-drugs) and antipsicotics for the treatment of comorbidities. Regular and frequent use of triptans and other symptomatic analgesic can cause chronic migraine and medication-overuse headache (MOH). The detoxification treatment of these subjects is necessary; the monitoring and follow-up are crucial to the success of the treatment. The hair is increasingly used to monitor adherence to pharmacological treatments and to document objectively the progress of detoxification and rehabilitation programs of drug addicts.
The method presented in this study allows the screening and quantification of 91 xenobiotics included triptans, benzodiazepines, z-drugs, analgesics, antiepileptic, antidepressants and antipsicotics, in hair.
Among sample preparation, 50 mg of hair samples were decontamined and pulverized with a ball-mill. The powder was incubated overnight in 2 ml of methanol, after addiction of IStd solution. Samples were purified by a new rapid procedure: after incubation, 1,5 ml of clear supernatant was added to QuEChERS dSPE Kit (150 mg $MgSO_4$, 50 mg PSA, 50 mg C18E). The final extracts were votexed and subsequently centrifuged at 5000rpm for 5 min; a total of 1 ml of the upper methanol layer was taken to dryness under a gentle strem of nitrogen. The residue was recostituted in 200 µl of mobile phase and analysed by LC-MS/MS with positive ionization MRM mode. Gradient elution was performed by a Kinetex 5µ Biphenyl 100A column.
Validation of the method was performed following the recommendations of international guidelines: the procedure was fully validated in terms of selectivity, linearity, limit of detection and lower limit of quantitation (LLOQ), accuracy, precision, recovery and matrix effect. Selectivity was evaluated by analysis of 10 certified drug-free hair samples. The limits of quantification (LOQs) ranged from 20 pg/mg to 50 pg/mg. Linearity was investigated in the range from LOQ to 2500 pg/mg with R^2 values of at least 0.990. The method offered satisfactory precision (RSD < 15%) and accuracy (80-120%) The matrix effect were within ±20% matrix for most analytes. Absolute recovery ranged from 60% to 95% for 75% of the analytes. The validated procedure has been applied to a total of 300 real hair samples collected from migraine patients who have given an informed consent before sample collection. The 3-cm proximal hair segment was used for the analysis.
The LC-MS/MS method was successfully validated and applied to hair sample of migraine patients whose the type and dose of drugs taken were known.

P131. PHOTOSTABILITY OF DRUGS OF ABUSE IN HAIR IRRADIATED IN A SOLAR BOX

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The concentration of drugs in hair may be altered by physical and chemical factors, among which environmental exposure. Previous studies on changes induced in hair by light exposure were performed in both model solutions and true positive hair irradiated by UVA and UVB lights.
In order to better understand the role and mechanisms of solar light exposure in decreasing hair concentrations of drugs, true positive hair samples, containing cocaine and metabolites, were irradiated in a solar box, reproducing the whole spectrum of sunlight.
Authentic hair samples that previously tested positive for cocaine were selected. Irradiation was performed in a Suntest CPS+ (Atlas, Linsengericht, Germany) equipped with a 1.8 kW xenon lamp and a glass filter (cut off 310 nm). 25 hair samples were collected of different natural colours (blond, brown, dark brown, black). Hairs, 5–7 cm long, were divided into two approximately identical strands: the former was put between two 5 x 5 cm optical glasses and exposed at 765 W/m2 (310-800 nm) for 48 hours to an endpoint corresponding to two months exposure under the sunlight. Hair samples were washed, extracted and analyzed by a validated method already proposed (Drug Test Anal 2014 , 6, 78-84) encompassing micropulverized extraction and liquid chromatography-high accuracy, high resolution mass spectrometry on an LTQ-Orbitrap. The % photodegradation was calculated as [100 *(drug concentration in the dark - drug concentration after exposure)/drug concentration in the dark.
The concentration ranges in the intact samples were 0,16 – 40,0 ng/mg and 0,05 - 19 ng/mg respectively for COC and BZE. 69 % of samples exhibited a decrease of COC in post-irradiation samples, with percent reduction from to 6 % to 72 % (mean 37,8 %); in 23 % of samples BZE decreased from 10 to 50 % of its initial concentration; in 46 % of samples BZE increased from 6 to 23 %; in 31 % of samples both COC and BZE contents did not vary. BZE increase was observed only in samples that exhibited COC decrease, suggesting that photodegradation of the parent compound generates BZE that remains incorporated into the hair shaft. For the 6 samples (23 %) that exhibited both COC and BZE decrease, the further degradation of BZE originally formed by photodecomposition of COC can be envisaged. No relation could be found with hair color or hair thickness.

When compared with our previous studies, when only specific UV components of sunlight (UVB at 311–312 nm and UVA at 365 nm) were used for irradiation, experiments in the solar box evidenced a similar percent of "degraded" hair samples (69 % solar box vs 62 % UVA/UVB) but a higher photodegradation yield of COC (mean 37 % vs mean 10 % respectively). The increase of concentration of a metabolite upon concomitant degradation of its parent compound highlights the peculiar role of whole sunlight and prompts for further studies, including other classes of compounds.

P132. ANALYSIS OF METHAMPHETAMINE, MDMA AND THEIR METABOLITES IN HAIR SAMPLES OF DRUG ABUSERS USING GC/MS/SIM

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Methamphetamine and 3,4 methylenedioxymethamphetamine (MDMA) are amongst the prevalent drugs of abuse in Singapore. The detection of such drugs in urine has always been the primary method to establish their consumption. However, in recent years, there has been a growing interest to use alternative matrices for drug detection and this includes hair analysis. The hair analysis will provide an alternative way to monitor the drug abuser relapse as it allows a wider detection window compared with urine analysis.
The primary objective of this study is to develop a gas-chromatography/mass spectrometry (GC/MS) method for the qualitative and quantitative analysis of methamphetamine, amphetamine, MDMA and 3,4 methylenedioxyamphetamine (MDA) in the drug abusers' hair samples.
Hair sample was washed twice using 5 ml dichloromethane, followed by 5 ml water and 5 ml methanol each, for about 1 min respectively. The decontaminated hair sample was then dried and finely cut with a pair of scissors. Corresponding deuterated amphetamines were added to 50 mg of the decontaminated hair as the internal standards. Digestion of hair was subsequently performed at 70 degrees Celsius using 1M NaOH and followed by liquid-liquid extraction using 1-chlorobutane/acetonitrile(4:1) solvent mixture. To prevent the loss of volatile amphetamines, 100 µl of 1% HCl in methanol was added to the mixture. Derivatisation of the sample extract was performed using 30 µl of trifluoroacetic anhydride (TFAA) and 30 µl of ethyl acetate. GC/MS analysis was performed using HP-5 capillary column and operating in the selected ion monitoring (SIM) mode.
Validation study of the method developed was performed using drug-free hair samples spiked with methamphetamine, amphetamine, MDMA and MDA reference materials. Standard calibration curves were obtained from drug-free hair samples spiked at the following concentrations in triplicates: 0.1, 0.2, 0.5, 1, 2, 5, 10 and 20 ng/mg. The calibration curves were found to be linear up to 20 ng/mg. The correlation coefficient of the calibration curve for each of amphetamine, MDMA and MDA was found to be 0.9995 while that for methamphetamine was 0.9998. The limit of detection (LOD) for all four drugs was 0.2 ng/mg. The limit of quantitation (LOQ) for each of methamphetamine, amphetamine and MDA was found to be 0.5 ng/mg and MDMA was found to be 0.2 ng/mg. Intra-day and inter-day precision study were also performed on all four drugs at spiked concentrations of 1, 2 and 5 ng/mg. The highest coefficient of variation (CV) observed for the intra-day study was 2.4% for amphetamine while that observed for inter-day study was 4.4% for MDMA. Extraction efficiency and interference studies were also performed. The method was applied to analyse the hair samples of 125 drug abusers who had abused amphetamines. The concentration range of the methamphetamine was found to be from 0.22 to 161 ng/mg, whereas, majority of the amphetamine, MDA and MDMA were found to be less than 5 ng/mg in the drug abusers' hair.
A GC/MS method for the qualitative and quantitative analysis of methamphetamine, amphetamine, MDMA and MDA in hair was successfully developed and applied to routine case analysis.

P133. DETERMINATION OF ANTICOAGULANT RODENTICIDES AND A-CHLORALOSE IN HUMAN HAIR BY ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY AND APPLICATION TO A REAL CASE

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Anticoagulant rodenticides are the largest group of pesticides used for control of harmful rodents. They are classified into two main groups, depending on their chemical structure: hydroxycoumarine and indandione rodenticides. Their fundamental mode of action is represented by the inhibition of the vitamin K epoxide reductase, which causes blood-clotting alteration, leading to extensive hemorrhages as the ultimate cause of death.
In this study, we developed a UHPLC-MS/MS for the simultaneous determination of 10 anticoagulant rodenticides (coumatetralyl, brodifacoum, bromadiolone, difenacoum, flocoumafen, coumachlor, acenocoumarol, coumafuryl, dicoumarol, warfarin), plus α-chloralose in human hair, with the scope of detecting potential chronological trace of poison exposure in clinical and forensic cases. The developed method was applied to a real case of alleged poisoning.
About 200 mg of hair was twice-washed with dichloromethane, then dried at room temperature and subsequently cut with scissors into 1–2 mm segments. An aliquot of about 50 mg was weighted. After the addition of 1 mL of methanol, the samples were incubated at 55°C for 15 h. Lastly, the organic phase was collected, and evaporated to dryness under a gentle stream of nitrogen and mild heating (25°C) The residue was dissolved in 100 µL of MeOH, transferred into vial, centrifuged at 4000 rpm for 10 min and 1 µL was injected into the UHPLC–MS/MS system. All analyses were performed on a Shimadzu Nexera 30 UHPLC-system interfaced to an AB Sciex API 5500 triple quad-

rupole mass spectrometer with a Turbo Ion source operating in the negative ion mode.
The optimized UHPLC-MS/MS method allowed the simultaneous determination of 10 anticoagulant rodenticides plus α-chloralose. The whole chromatographic run, comprehensive of the time required for column re-equilibration, was completed in 8.5 min. Retention times ranged between 1.39 min (coumafuryl) and 4.33 min (brodifacoum). In the real case, a segmental hair analysis was performed. Difenacoum was detected in the first hair segment (0-3 cm) at the concentration of 2.9 pg/mg, while α-chloralose was detected at the concentration of 85 pg/mg. The two remaining, consecutive segments (3-6 and 6-9 cm) showed traces of difenacoum (below the LOQ) and low but quantifiable levels of chloralose (29 pg/mg and 6 pg/mg, respectively).
An UPLC-MS/MS method for the simultaneous determination of 10 anticoagulant rodenticides and α-chloralose in human hair was developed and validated. The method proved to be simple, accurate, rapid and highly sensitive, allowing the simultaneous detection of all compounds. Finally, the method was applied to a real case of difenacoum and α-chloralose poisoning and proved sensitive enough to detect the occasional exposure to both analytes.

P133. SIMULTANEOUS DETERMINATION OF LSD AND 2-OXO-3-HYDROXY LSD IN HAIR BY LC-MS/MS

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Lysergic acid diethylamide (LSD) is one of the most potent and commonly used hallucinogens. The detection of LSD in biological matrices is challenging because of the low oral dose (approximately 20-200 µg) and extensive metabolism. Hair has gained significant attention as a fundamental biological specimen for drug abuse in the field of forensic toxicology. However, limited data exists on the analysis of LSD and its metabolites in hair compared with those in urine or blood.
In this study, a sensitive and reliable method based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) was established and validated for the simultaneous determination of LSD and its metabolite, 2-oxo-3-hydroxy-LSD (O-H-LSD), in hair. Furthermore, the method was applied to authentic hair specimens from forensic cases.
The hair was cut and weighed accurately (ca. 10 mg), and methanol (2 mL) was added to it. After fortifying an internal standard, the hair samples were incubated at 38 °C for 15 h in dark room. The extract was evaporated and reconstituted prior to LC–MS/MS analysis. The mass spectrometric analysis was operated in electrospray ionization (ESI) positive ion mode. Validation of the analytical method was performed and hair specimens from two suspected LSD users were analyzed for the documentation of LSD intake.
Limits of detection (LODs) in hair were 0.25 pg/mg for LSD and 0.5 pg/mg for O-H-LSD, respectively. Method validation results showed good linearity and acceptable precision and accuracy. The developed method was applied to authentic specimens from two legal cases of LSD ingestion, and allowed identification and quantification of LSD and O-H-LSD in the specimens. In the two cases, LSD concentrations were 1.27 and 0.95 pg/mg, respectively; O-H-LSD was detected in one case, but its concentration was below the limit of quantification.
The developed method provides good selectivity and sensitivity for both analytes at low concentration levels using a small amount of sample. Furthermore, this method was successfully applied to authentic samples from legal cases, and can be used for routine analysis for documenting LSD use in the field of forensic and clinical toxicology.

P135. IS HAIR A SUITABLE BIOLOGICAL MATRIX FOR CANNABIDIOL DETECTION?

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Cannabidiol (CBD), one of the cannabinoids presents in Cannabis sativa, may show antipsychotic and anxiolytic properties as well as neuroprotective activity in human. Recently, Brazilian Health Surveillance Agency has reclassified cannabidiol from banned to controlled drug. It has been prescribed to patients who present syndromes that lead to spasms and epilepsy as well as are resistant to traditional treatments.
The aim of the study was to detect THC and CBD concentrations in hair samples of 100 volunteers from a public health service in Ribeirão Preto city, Brazil using LC-MS-MS and also to analyze 50 samples of street Cannabis sativa plant seized by the local police using GC-MS.
At first, intact hair samples were placed in a glass tube and decontaminated with 3 ml dichloromethane followed by 3 ml methanol. After solvent addition and vortex mixing (30 s), the solvent was removed with a pipette. After the second washing, solvent residues were removed from hair under a mild nitrogen stream at 40°C for 15 min. Hair was then cut with scissors into small pieces (1 – 2 mm segments) and 10 mg aliquots (approximately) were placed in an Eppendorf tube and were sent to a private lab in São Paulo (Chromatox), that performed the analysis using SPE and LC-MS/MS (LOQ = 0.02 ng/mg). A qualitative analysis of street Cannabis was performed using 240 mg (± 2%) plant material extracted with 10 mL methanol/chloroform (90:10) and sonication for 30 min. A 100 µL aliquot was diluted in 10 mL methanol. Then, 100 µL of methanol dilution was evaporated, ressuspended with ethyl acetate and derivatized with MSTFA (70°C/20 min). 1 µL was injected into the GC-MS system.
A total of 100 hair samples were analyzed using LC-MS/MS. THC was detected in 42 samples but only 22 samples were positive, according to the confirmation cut off recommended by SOHT. THC concentrations ranged from 0.05 to 0.6 ng/mg (mean of 0.13 ng/mg). However, CBD was not detected in any sample. Considering this finding, 50 street Cannabis plant samples were analyzed to assess their CBD concentration. These plant extracts were kindly provided by the Narcotics Investigations Laboratory of Forensic Expertise Center of Ribeirão Preto, Brazil. The proportion

of CBD to THC in the plants ranged from 0 to 0.019 (mean of 0.003 ± 0.003). Considering that THC concentration found in hair ranged from 0.05 to 0.6 ng/mg, a theoretical CBD concentration in hair could be of 0.00015 to 0.0018 ng/mg. These values require a very sensitive detection method for Cannabinoid hair testing.
If an extremely sensitive detection method, capable of analyzing such low concentrations of CBD is not available, hair will not present a suitable biological matrix for CBD identification. And considering that CBD levels in Cannabis plant seem to decrease worldwide, it is crucial to continue the development of satisfactory, robust and high throughput techniques which will enable a reliable detection of CBD concentrations in hair samples to, consequently, evaluate chronic exposition of this drug in the patients.

P136. AMPHETAMINES AND CANNABINOIDS TESTING IN HAIR: EVALUATION OF RESULTS FROM A TWO-YEAR PERIOD

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Hair testing enables a retrospective identification of long-term drug use, thus becoming a routine procedure in Forensic Toxicology. As a result, large datasets of drug concentration in hair are available for statistical analysis, which is a very suitable approach to obtain insights about the target population and to improve the interpretation of quantitative results.
This study aims to overview a series of amphetamines and cannabinoids tests performed on head hair samples in our laboratory between 2013 and 2014 and to assess the ratio of positive results in relation to sex, age, hair colour, dyeing and length of the tested samples. This will increase the knowledge of drug abuse patterns within an extended area of Spain.
Hair samples were tested for stimulant phenylalkylamine derivatives (amphetamine, methamphetamine, MDMA, MDA and MDEA) and/or cannabinoids (THC and cannabinol) using a method accredited to ISO/IEC 17025. The samples were washed in dichloromethane, dried, weighed, and subjected to alkaline digestion in NaOH solution. Cannabinoids were extracted from hair samples with n-hexane/ethyl acetate; amphetamines were extracted in solid phase and derivatized with pentafluoropropionic anhydride and ethyl acetate. The reconstituted extracts were injected onto the GC-MS/EI for selected-ion monitoring. All the analytes were identified and quantified (cannabinol was only identified). The test results were interpreted according to the confirmation cut-offs proposed by the Society of Hair Testing, i.e. 0.20 ng/mg for amphetamines and 0.05 ng/mg for THC.
21,94% of the 2954 hair samples tested for phenylalkylamine derivatives were positive for one or more substances. Amphetamine showed the most frequent positive result (16.38%), followed by MDMA (12.09%), while methamphetamine was positive in only 0.44%. The amphetamine/MDMA combination was found positive in 6.60% of the cases. A total of 3178 samples were tested for cannabinoids, resulting in 53.40% positive for THC and cannabinol. Simultaneous tests for phenylalkylamine derivatives and cannabinoids were performed in 2931 of the samples; 14.94% of them were positive for THC, cannabinol, and one or more amphetamines. Significant differences in the ratio of positive results were found depending on the tested drug: amphetamine only showed differences in relation to dyeing, MDMA in relation to sex and age, and THC in relation to sex, age, hair colour (lower ratio in white/grey hair than in brown/black one, probably due to differences in melanine), and length of the tested samples (only 3.0-5.5cm-long proximal segments showed similar ratio to the global ratio obtained from all the samples, whose length ranged from 1.0 to 7.5cm). Low, medium and high ranges of concentration were estimated for each drug by using the interquartile range of quantitative results. According to our results, the use of THC and MDMA vary with age and sex in an extended area of Spain, while the use of amphetamine appears to be independent of these variables. On the other hand, the results of THC in hair could be influenced by the length of the tested segment; therefore, a consensus regarding the hair length between 3.0 and 5.5 cm for THC testing should be reached.

P137. HAIR PREPARATION FOR CANNABINOID ANALYSIS: HOW CUTTING AND GRINDING AFFECTS RECOVERY ?

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Since the beginning of hair analysis in the early 90's, there has been a lot of different hair preparations used before the extraction process for drug of abuse screening. Grinding was mostly used in the beginning of the hair analysis era but owing to the significant loss of material in the ball mill this technique was soon replaced by a simpler cutting procedure. With this type of technique hair is cut directly in a vial as many times needed to achieve approximately 1 mm segments. Although it seems obvious that differences in extraction could exist between powdered hair and segmented hair, there are very few articles reporting on the comparison of the two methods (grinding and cutting) on particular analytes. Recently several articles reported on the higher concentrations of EtG obtained in hair after using grinding procedure.
Two different hair preparation procedures (grinding and cutting) were evaluated for the analysis of cannabinoids.
A method was developed for the analysis of THC, cannabinol, cannabidiol and carboxy-THC. Hair strands were decontaminated twice during 1 min in a methylene chloride bath. Hair was dried before being either powdered in a grinding jar using a ball mill or cut with scissors. Twenty mg of hair were weighted and spiked with deuterated internal standards. Hair hydrolysis was obtained with 1mL of 1M sodium hydroxide. Extraction was performed twice, first basic extraction with heptane/ethylacetate allowed to retrieve THC, cannabinol and cannabidiol. Then the reminding aqueous phase was acidified and extracted again with heptane/ethylacetate. Both organic phases were evaporated to dryness. The phase from basic extraction was injected as this in the LC-MS-MS system. The acidic

extraction phase was derivated with dansyl chloride to improve the sensitivity for carboxy-THC.
Linearity was achieved from 20 to 5000 pg/mg for THC, cannabinol and cannabidiol and from 0.2 to 50 pg/mg for carboxy-THC. Ten different hair samples from cannabis users were investigated. Mean concentrations of THC were 454,2 pg/mg and 151,4 pg/mg respectively for cut and grinded hair. For carboxy-THC the mean concentrations were 9.5 pg/mg and 5.6 pg/mg for cut and grinded hair. Concentrations measured in grinded hair were consistently lower than the ones measured in cut hair for the four analytes except for 1 subject whose concentrations were similar with both preparations. The method developed allows achieving the required LOQ for carboxy-THC, THC, cannabinol, and cannabidiol analysis in hair. The method is quite complex but does not require the use of MS3 like recently published techniques for LC-MS-MS analysis of carboxy-THC. There is a major difference in the results obtained between the two preparations. Concentrations are higher for all analytes when hair is cut. We are currently investigating the possible causes for this result such as adsorption of the cannabinoids on the grinding jar material.

P138 – P275 **SESSION III and IV**
(changing panels on Wednesday morning)

P138. **METHOD DEVELOPMENT FOR DETERMINATION OF SOME NEW STIMULANT DESIGNER DRUGS FROM BLOOD AND URINE.**

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Classical amphetamines (amphetamine, MA, MDA, MDMA, and MDEA) and more stimulant designer drugs are determined by GC-MS in our laboratory following extraction by toluene and derivatization by heptafluorobutyric anhydride (HFBA). Under these conditions 8 substances (4-methylbuphedrone, 4-MEC, alpha-PVP, methoxethamine, buthylone, MDPV, MDPBP, 2C-B) of 35 measured produce a peak shape at lower concentrations what is difficult to evaluate.
The aim of this work was to develop a new method to measure these 8 substances in blood and urine samples which fits the GC-MS conditions used for determination of other illicit drugs and medicines in our laboratory.
200 µl urine or blood sample, 50 µl bicarbonate buffer (pH = 11), 10 µl ISTD and 0.5 ml acetonitrile (ACN) was mixed and vortexed for 15 seconds. Then, cca. 400 mg $(NH_4)_2SO_4$ was added, the mixture was stirred and centrifuged (2500 rpm, 5 min.). 400 µl of the upper phase was evaporated and resolved in 75 µl acetonitrile (ACN). To 30 µl of the ACN solution 15 µl N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) was added and derivatized at 80oC for 30 minutes and measured by GC-(EI)-MS in SIM mode. The GC-MS conditions are previously described.
The new method fulfilled the validation criteria: linearity: $R^2 \geq 0.98$; intra- and inter-day precision: bias and RSD < 15%; selectivity LOQ : matrix ratio ≥ 3 for target ions and ≥ 2 for qualifiers; stability: < 20% degradation within 24 hours. For 4 of the 8 substances (buthylone, methoxethamine, MDPV, and MDPBP) the LOQ values decreased from 40 to 20 ng/ml. The shape of the peak of the target and qualifier ions was symmetric and easy to evaluate also at the lower concentrations. The new method allows a more precise and, for some substances, more sensitive determination of stimulant designer drugs in biological samples. It also provides a new alternative of method development for the new stimulant designer drugs appear in the illicit market.

P139. **A COMPREHENSIVE SCREENING OF ILLICIT AND PAIN MANAGEMENT DRUGS FROM WHOLE BLOOD MATRIX USING SPE AND LC/MS/MS**

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Both in Therapeutics Drug Monitoring and Toxicology Screening, time is crucial in order for the generated data to be still useful. Complicated biological matrices make this goal very tough to achieve in a reliable way.
Utilizing advanced sample preparation techniques we simplify a complicated matrix to allow for a fast and successful multi-component analysis by LC/MS/MS.
Six pretreatment options for whole blood were investigated. A 5% zinc sulfate and acetonitrile combination worked for most classes. However, there was not a single pretreatment procedure that worked well for all classes of compounds. The supernatant was loaded onto the Strata-X-C 30mg/3mL cartridges. Salts and most endogenous components were removed using a two-step, 0.1% formic acid and 30% methanol, wash. Analytes were eluted using 2x500 uL of ammoniated IPA/ethyl acetate solution. Samples were then acidified and evaporated to dryness then re-suspended in mobile phase. The chromatographic separation was performed on a Kinetex 2.6um Biphenyl 50x3.0mm column. The mobile phase was 0.1% formic acid in water and 0.1% formic acid in methanol. The detection was achieved on an AB Sciex Triple Quad 4500 and/or a 4000 QTrap LC/MS/MS system. All analytes were detected under positive polarity and MRM scan function using two mass transitions. A MRM-IDA-EPI combination scan was used on a small sample set to verify the analyte purity.
A detection limit of 10ng/mL was achieved for all tested analytes using this method. Although a full calibration method was not vigorously tested, the upper end of the calibration range was deemed to be 1000ng/mL. Early eluting analytes were well separated from the ion suppression zone by a retention factor of two, or by three times the dead volume. Isomeric/isobaric compounds were completely resolved by at least a factor of 2.
The described procedure provides a simple and reliable solution to screen a wide range of illicit and pain control compounds from a complicated matrix.

P140. **SIMULTANEOUS QUANTIFICATION OF 15 SYNTHETIC CANNABINOIDS AND METABOLITES IN BLOOD BY LIQUID CHROMATOGRAPHY-HIGH RESOLUTION MASS SPECTROMETRY**

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Synthetic Cannabinoids (SCs), commonly known as "synthetic marijuana" are marketed under various brand names such as "Spice", "K2". They are often sold as herbal incense or air fresheners on websites, in head shops and labeled "not for human consumption" to mask their intended purpose and avoid control law. To analyze SCs in seized materials and biological matrices, several mass spectrometry techniques have been used, gas chromatography–mass spectrometry (GC–MS), liquid chromatography–tandem mass spectrometry (LC–MS/MS). But high resolution

techniques such as liquid chromatography-high resolution mass spectrometry (LC-HRMS) provide more sensitivity and selectivity than the others.
The aim of the present study was the validation of a liquid chromatography-high resolution mass spectrometry (LC-HRMS) method for the simultaneous identification and quantification of 15 synthetic cannabinoids and their 20 metabolites in blood samples. The method was applied to authentic forensic cases submitted to the Toxicology Department of The Council of Forensic Medicine in Istanbul by the judicial authorities.
Samples were prepared with solid-phase extraction method using OASÐS HLB cartridges. Gradient elution was performed by Accucore Phenyl Hexyl (100mmx2.1mm, 2.6µ particle size) analytical column and Δ9-THC-d3 was used as internal standard (IS). The Q Exactive mass spectrometer was operated in the positive ionization mode. The mass spectrometer acquired a targeted-MSMS scan at a resolution of 35.000
We developed a sensitive and specific method for the determination of 15 synthetic cannabinoids and 20 metabolites in blood by liquid chromatography high resolution mass spectrometry. The method was validated according to the international guidelines in terms of selectivity, linearity limits of quantification (LOQs), intra-assay and inter-assay precision and accuracy, recovery,matrix effects, process efficiency. Validation of the method was performed by the use of spiked drug-free blood samples.All analytes were found to be linear in range with the correlation coefficient greater than 0.998 for the samples with 0.1 to 50 ng/mL concentrations in blood. The method was applied to authentic forensic cases submitted to the Toxicology Department of The Council of Forensic Medicine in Istanbul by the judicial authorities.
An LC-HRMS method for the highly specific and sensitive quantification of 15 synthetic cannabinoids and metabolites in blood, has been validated. The method is considered as reliable and suitable for the analysis of routine forensic samples.

P141. DEVELOPMENT OF A METHOD FOR DETERMINATION OF COCAINE, COCAETHYLENE AND NORCOCAINE IN HUMAN BREAST MILK USING LIQUID PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Most licit and illicit substances consumed by the nursing mother might be excreted in breast milk, which may cause potential short and long term harmful effects for the breastfed infant. Even though there is an evident increase in the knowledge of psychoactive substances during lactation, there is still limited information about the levels at which these substances are excreted by breast milk as well as several unanswered questions concerning their possible effects in the infant during nursing period. The extraction of substances from breast milk represents an analytical challenge due to its high protein and fat content as well as the fact that its composition changes during postpartum period.
The aim of the present study was to develop a method for detection of the active substances: cocaine (COC), cocaethylene (CE) and norcocaine (NCOC) in human breast milk. The analyses were performed by using liquid phase microextraction (LPME) followed by gas chromatography mass spectrometry (GC-MS).
Prior to extraction, 0.5 mL breast milk fortified with 50 µL of the deuterated standards at 1µg/ mL (COC-d3, NCOC-d3 and CE-d3) were diluted with 0.5 mL of borate buffer (pH 9.0). For each extraction procedure a new 8 cm length polypropylene hollow fiber was immersed in n-octanol and the excess of solvent was removed by sonication in water for 10s. After immobilization, the acceptor solution (HCl 0,4M) was injected into the hollow fiber, which was then inserted into the sample solution vial. Posteriorly, this was submitted to vortex vibration for 30 minutes at 2400 rpm. The solution collected from inside the fiber was dried under N_2 flow at 40 °C. This was derivatized at 60ºC for 30 minutes with 25 µL BSTFA-1%TMCS and 25 µL acetonitrile. Once this solution was cooled down to room temperature, 1 µL was injected into the GC-MS.
Validation was performed working on spiked human breast milk samples. The limits of detection (LoD) and quantification (LoQ) were of 6 and 12 ng/mL, respectively, for all analytes. Calibration curves were linear over a concentration range of 12.0 ng/ml to 1000 ng/ml (R^2 = 0,99). Selectivity was evaluated by analyzing 20 negative breast milk samples, which showed no interferences at the retention times of interest. Within-run and between-run precision, have been analyzed at three different concentrations (40, 400 and 850 ng/mL) and the result obtained has been less or equal to 15 as % relative standard deviation. Bias (at 40, 400 and 850 ng/mL) ranged from 3 to 18%. Inactive metabolites, such as anhydroecgonine methyl ester and benzoilecgonine, were not included. One out of three real samples analyzed, revealed a positive result in a concentration lower than the LoQ.
This method has shown to be a reliable alternative for the determination of cocaine, cocaethylene and norcocaine in human breast milk. LPME extraction procedure has proven to be a rather promising, low cost and environmental-friendly technique for the purpose of this study. Also, there is an urge in studying such substances as they seem to be harmful to the breastfed infant.

P142. CHRONIC LEAD POISONING BY INTRAMUSCULAR INJECTION OF KOHL: A CASE STUDY

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Kohl is a gray or black powder, known since immemorial time as a symbol of beauty (eye cosmetics). Its use as a traditional remedy is not rare in Algeria. Thus, it is used against the eye infections and as a treatment of leishmaniasis. This powder is used also to accelerate the fall of the newborn's umbilical stump and for hygienic measures and to stop bleedingafter boy's circumcision. The criminalization of this powder often contains lead in lead poisoning is not new, but its use is usually limited to a local application in contrast to the case which is the objective of this study.
The aim of this presentation is to report the first case of lead poisoning by a voluntary intramuscular injection of khol used as a traditional remedy.
It is a study of clinical data and toxicological analysis reported in the medical records of a patient admitted to the medical neurology department.
A 42-year-old Algerian man, teaching in a school, presented to medical neurology department for a sever sub acute sensory-motor polyneuropathy, affecting all four limbs. In his medical history, there is a notion of muscle pain since the age of 22 and impotence to which he had injected repeatedly kohl mixed with water into a muscle for two years. Several surgical procedures were required to evacuate the abscess formed in the buttocks and multiple fistulae in the left thigh. An examination was performed, the patient had total motor deficit, areflexic and a sensory disorder in all modes of the four limbs, a mental depression, insomnia, anorexia, weight loss, cough and constipation with blackish stool and urine. Lead poisoning is then suspected, it is confirmed by a blood lead level of 138,2 µg/dL. Iron deficiency anemia (microcytic, hypochromic), discrete hemolysis and basophilie stippling of red blood cells are found. The standard radiograph of the pelvis (performed before surgery abscesses) reveals a historical picture made of an opaque casting projecting the right iliac bone, some bone demineralization and calcified streaks in soft tissue.The introduction of a chelator treatment with 2,3-dimercaptosuccinic acid (DMSA)for 20 days has reduced blood lead levels to 15,5µg / dl and improve the patient's clinical condition. In parallel, proper rehabilitation allows a gradual improvement in the motor plan. Other therapeutic cures were necessary because of a likely redistribution from the bone.Lead rate in a sample of offending khol was 0,05%.
The involvement of kohl in chronic lead poisoning is not new although it remains unknown to the general public and even health personnel, but the use of the intramuscular route is a first in history. Also, this case put an update on the inappropriate use of traditional treatment and it heavy health consequences.

P143. DRUG AND SUBSTANCE ABUSE IN REFRACTORY EPILEPSY

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Seizures often occur in substance abusers.
to study the etiology of non-response to antiepileptic drugs by estimating their serum levels and screening of drugs and substance abuse in patients with resistant epilepsy
Materials & Methods; This study was conducted in epilepsy out patient clinic, neurology department, Mansoura University Hospital. After exclusion of those with symptomatic epilepsy or non compliance to treatment or with epilepsy other than generalized tonic-colonic seizures, 924 patients with resistant epilepsy were included. They were subjected to:
-Toxicology screen for detection of drug and substance abuse by analysis of urine and blood samples.
-Measurements of the level of antiepileptic drugs in the blood (carbamazepine, valproic acid, phenytoin).
All assays run on the system use of homogenous immunoassay technique EMIT (Enzyme Multiplied Immunoassay Test) and confirmed by GC/MS (gas Chromatography/Mass Spectrum). Confirmed Positive results for drugs and substance abuse were found in 246 of 924 patients (26.62%) by GC/MS. Cannabis was the first abused drug (29.27%), opiates was the second drug abused by patients (21.95%) followed by alcohol (17.88%), benzodiazepine (16.26%) tricyclic antidepressants (8.54%) and finally barbiturate constituted (6.1%). Only 17 patients show serum level of antiepileptic drugs (carbamazepine, valproate and phenytoin) within therapeutic range, but 169 patients' levels were below it and 60 patients with levels above it.
It is important to note that the seizure problems related to recreational drug use are usually caused by acute intoxication, in contrast to the withdrawal seizures encountered in subjects who have been abusing alcohol, benzodiazepines or barbiturates. It is important to recognize that alcohol predisposes to many medical and metabolic conditions that can mimic or cause seizures.

Poster abstracts

P144. BACLOFEN OVERDOSE: TOXICOKINETIC STUDIES IN TWO CASE REPORTS

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Baclofen is a centrally acting gamma-aminobutyric acid agonist, currently used in France for the treatment of patients dependent in alcohol and non-respondent to others treatments. We describe two case reports of patients admitted in intensive care units.
Describe elimination kinetics of baclofen in two case reports with patients who received or not depurative treatments.
Supposed baclofen doses ingested were 250 mg in case A and 3500 mg in case B. After hospital admission and during 6 days in case A, 9 plasma samples and 4 urine samples were analyzed. In case B during 21 days, 34 plasma samples and 14 urine samples were analyzed. To 100 µL of plasma (or 100 µL of urine diluted 1/100) samples, baclofen-d4 (10 ng) was added and precipitated with 300 µL of acetonitrile containing 5% formic acid. Chromatographic separations were achieved using an Accucore PFP column (100 x 2.1 mm, 2.6 µm, Thermo Scientific) with gradient elution with water and acetonitrile both containing 0.1% formic acid. Analysis were performed on a LCMSMS method with a Quantuum Ultra apparatus (Thermo Fisher Scientific) with electrospray source ionisation in positive mode.
The elimination of baclofen in case A undergoes a first order kinetics with a plasma half-life of 11.1 h and an estimated maximal concentration of 793 ng/mL (t = 2 h). After three days, the toxic was eliminated without depurative treatment (plasma concentrations < 10 ng/mL). Presence in urine samples was observed during six days. In case B, maximal concentration was estimated at 6545 ng/mL (t = 2 h) and elimination half-lifes observed during first days were 8.4 h and between 6.7 and 7.2 h after the three hemodialysis. After ten days and an activated charchoal treatment, plasma concentrations finally decreased and half-life was 38.7 h. Before charcoal administration, plasma concentrations of baclofen decreased and increased numerous time. After this treatment, plasma concentrations definitively decreased and urine concentrations began their diminution.
In the two cases, half-lifes were longer than 2-6 h described under therapeutic treatments. In case A, hemodialysis was not necessary for the toxic elimination. In case B, secondary increases of baclofen levels, a finding also reported by other authors were probably attributed to the prolonged intestinal absorption of baclofen. Usually, hemodialysis is described as a reasonable treatment modality in patients with accidental baclofen overdose. In case B, hemodialysis were not effective and the effect of charchoal treatment in a second time, allows to suppose the presence of gastric bezoars during ten days which could explain absorption observed after hospital admission. Despite the severe clinical manifestations of baclofen intoxication, the outcome was good in the two cases.

P145. LC-MSMS QUANTITATION OF 25B-NB2OME IN PLASMA AND URINE.

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A validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was established for the quantitation of 25B-NB2OMe concentrations in plasma and urine.
To establish a quantitation method for the measurement of 25B-NB2OMe in samples received from the Emergency Department at Christchurch Hospital from suspected overdose patients. The method needs to be robust and accurate with a quick turnaround time and low sample volume.
25D-NB2OME was used as internal standard as the samples were screened first and none of them contained this compound. For plasma, 50 µL of plasma was added to 75 µL of internal standard (25D-NB2OME 10 µg/L in acetonitrile), vortex mixed and centrifuged at 18000 x g for 5 min to precipitate the proteins. A 100 µL aliquot of clear supernatant was mixed with 300 µL of water. For urine, 100 µL of urine was added to 100 µL of internal standard and 800 µL of water followed by mixing. For both plasma and urine, 20 µL was injected into the LC-MS/MS system (Agilent 1290 Infinity Series High Performance Liquid Chromatograph connected to an Agilent 6460 Series Triple Quadrupole Mass Spectrometer; Agilent Technologies, Santa Clara, CA, USA). A Poroshell 120 EC C18 2.7 µm, 50mm × 3.0mm column (Agilent Technologies) was used for separation under gradient elution with acetonitrile and 0.2% formic acid and 10 mmol/l ammonium formate in water. The total analysis time was 5 min. Mass spectrometric detection was in the positive mode with 25B-NB2OMe monitored at 380.1/121.1 and 380.1/91.1 eluting at 2.06 min and 25D-NB2OMe at 316.1/121.1 and 316.1/91.1 eluting at 2.01 min. Linear calibration curves were used for the range 0.1-10 µg/L in plasma and 1-200 µg/L in urine.
Plasma concentrations in samples from 10 patients admitted to the Emergency Department ranged from <0.1 µg/L through to 10.9 µg/L, while urine concentrations ranged from 1.0 µg/L through to 178 µg/L. Interday precision and bias for quality control samples (1 and 10 µg/L in plasma, 10 and 100 µg/L in urine) were within 8% and within 18% at the lower limit of quantification (0.1 µg/L in plasma, 1 µg/L in urine). Recoveries were complete and matrix effects, ranging from 71-87% were accounted for by the internal standard. Patient samples were analysed in duplicate with a deviation of <10% from the mean.
The method has proven to be effective in the quantitation of 25B-NB2OMe in both urine and plasma from individuals suspected of ingesting this substance. Low sample volume was achieved and the short sample run time of 5 minutes was in keeping with the requirements of the analysis.

P146. THE PREVENTIVE MAINTENANCE OF LATE COMPLICATIONS OF THE ACUTE ACETIC ACIDE POISONINGS

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Critical states in acute poisoning are always accompanied by severe disorders of hemodynamics and microcirculation disturbance. This is especially true for patients with severe acute acid poisoning, when extensive chemical burns of the gastrointestinal tract, a high level of hemolysis leads to a sharp increase of vascular permeability. Hypostasis, nekrosis, purulent mediastinitum and stricture of walls of a gullet after a chemical burn are terrible complications of sharp poisonings by acetic acids and it lead patients to physical inability. In the subsequent, the sick volume regenerative operations are required.
Evaluate the effectiveness of the method developed for the prevention of late complications of acetic acid poisonings.
Materials and methods. This study consisted of 96 patients hospitalized in 2010-2014. with acetic acid poisoning and severe (III) degree of chemical burns of the esophagus and stomach. All victims were divided into two groups: I group (basic) - 66 patients, who in 2010 received an advanced algorithm of intensive therapy including the drugs improve microcirculation (HES 130-volustim), high-dose corticosteroids for 25-28 days, injections for cytoflavin 20.0 ml a day, actovegin to 10.0 ml / 2 times a day, from the moment of receipt and within 15-20 days. From 25-30 days we injected intramuscularly absorbable drug hyaluronidase 64 IU 2 times a day for two weeks The comparison group (II) consisted of 30 patients were admitted in 2010, and received conventional therapy. All patients was studied their oropharynx, esophagus and stomach, by laryngoscopy and endoscopy, on the 1-th day and in the dynamics, as well as pathomorphological study of mucosae of the esophagus after 3 months
Results: At the first day in both groups during endoscopic study has been a sharp hyperemia, swelling. The mucosa of the oropharynx and esophagus was covered with a thick fibrinous plaque. In dynamics, the basic group on the 25-30th day the disappearance of fibrinous deposit, and redness and swelling of the mucous membranes is decreased. The laryngoscopic and endoscopic picture of the II group of patients to the 7-th day kept all signs fibrinous inflammation. In the common number of patients of the I group, only 11 had developed scar stricture of the esophagus (16,6%). Among the II group stricture was formed in 22 (73,3%) patients. The study of esophageal biopsy in the month after the burn is showed that patients in the I group observed microscopically ordering layering of the epithelium, with little plots without epithelium. Morphological pattern of the esophageal mucosa of patients in the II group was characterized by atrophic changes in the squamous epithelium.
Conclusion: The introduction of an improved algorithm of intensive therapy in the complex treatment of acetic acid poisoning is effective in the prevention of late complications.

P147. A REAL CASE OF INTOXICATION ASSOCIATED WITH THE RECREATIONAL USE OF DIPHENIDINE

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Diphenidine (1-(1,2-diphenylethyl)piperidine) is a dissociative anesthetic drug usually sold online as a research chemical. Anecdotal reports describe that high doses of diphenidine produce stimulation, euphoria, shifts in perception of reality, distorted sensory perceptions, hallucinations and transient anterograde amnesia. Some users reported undesired effects including tachycardia, hyperthermia, panic attacks, and, after ingestion of higher doses, seizures requiring hospitalization.
In this study, we report a case of acute toxicity after oral diphenidine use. A 30-year-old Caucasian male was hospitalized because he was affected by a severe state of hallucination and agitation. The physical examination revealed tachycardia (160 bpm) and enhanced body temperature (38.0° C). Laboratory analysis revealed acidosis and increase of creatine kinase. A small, transparent plastic bag labeled as diphenidine was found on him, containing 1 gram of white crystalline powder.
The powder was analyzed in GC-MS (full-scan acquisition) and confirmed to be diphenidine. The abuse of diphenidine was corroborated by its detection in plasma and urine, using a GC-MS method developed on-purpose. Hair specimen was analyzed using a Agilent 1290 Infinity UHPLC-system interfaced to an AB Sciex API 4500 QTrap mass spectrometer with a Turbo Ion source operating in the positive ion mode.
The diphenidine concentration in urine was 0.63 mg/L, while its concentration in plasma was 0.31 mg/L. Other findings in plasma included: diazepam (0.38 mg/L), nordiazepam (0.007 mg/L), lormetazepam (0.002 mg/L), lorazepam (0.005 mg/L), delorazepam (0.04 mg/L), midazolam (0.21 mg/L), haloperidol (0.004 mg/L), and methylphenidate (0.003 mg/L). Hair analyses revealed a history of heavy diphenidine abuse (concentration: 5.3 ng/mg), although external contamination from powder handling could not be excluded.
Both circumstantial elements and toxicological results testify the occurrence of acute intoxication ascribed to the recreational abuse of diphenidine. The abuse of diphenidine likely occurred in association with methylphenidate. The simultaneous detection of lormetazepam, lorazepam and delorazepam in blood is ascribable either to intake of lormetazepam and delorazepam, or alternatively from the metabolism of the designer benzodiazepine named diclazepam. This is the first case reported on the Italian territory of diphenidine abuse.

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P148. **POLYDRUG ABUSE REVEALED BY COMPREHENSIVE UHPLC-HR-TOFMS**

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Drug abuse, particularly polydrug abuse, is a significant health problem with the most severe consequences being overdose morbidity and mortality. In Finland, the number of drugs of abuse findings in post-mortem material has increased, buprenorphine being the most prevalent. Polydrug abuse has been shown to increase the risk of premature termination of opioid maintenance treatment (OMT), which in Finland is provided by public healthcare and private foundations. Moreover, the range of toxicologically relevant compounds is continually changing due to the emergence of new psychoactive substances (NPS). This sets considerable demands for drug screening methods. The whole range of abused drugs cannot be determined with current immunoassays, necessitating a more comprehensive method. An imminent estimation is that the number of people with drug-related health problems is increasing as the abuse of medicinal drugs and NPS is becoming more prevalent.
Our objective was to compare the drug abuse patterns between drug abusers attending treatment, outside treatment, and those post-mortem cases in which NPS were found.
The study material consisted of 278 clinical urine samples provided by three healthcare centers (two rehabilitation clinics for opioid dependent patients and a harm reduction unit (HRU) specialized in counseling drug abusers using intravenous drugs), and 27 post-mortem samples collected at medico-legal autopsies. The clinical samples were from drug abusers undergoing (n=200) or in queue (n=13) for OMT, in drug withdrawal therapy (DWT, n=31), and from untreated drug abusers with self-reported NPS-abuse irregularly visiting HRU (n=34). The post-mortem samples were all NPS-positive samples from a one-year period (2014). After solid-phase extraction the samples were subjected to UHPLC-HR-TOFMS analysis with simultaneous acquisitions of high and low collision energy. Compounds were identified by a post-targeted database search with preset reporting criteria. The method covered various classes of NPS, conventional drugs, and prescription drugs. The identified drugs were sorted into eight drug groups (benzodiazepines, buprenorphine, cannabis, amphetamines, opioids, NPS, pregabalin and gabapentin, and cocaine).
In NPS-related post-mortem cases, typical concurrent findings consisted of a benzodiazepine, amphetamine, and buprenorphine. During 2014, phenazepam was the most common NPS in post-mortem cases and alpha-PVP in other groups. Usually an NPS finding was always accompanied by several other drugs. Cocaine and 6-monoacetylmorphine were only found in post-mortem cases. The most frequent drug abuse pattern for an untreated drug abuser included amphetamine, cannabis, buprenorphine, benzodiazepine, and alpha-PVP, and the abuse of pregabalin was common. NPS and pregabalin abuse was always related to drug co-use. The most typical drug abuse pattern among drug abusers in DWT and in OMT-queue included buprenorphine and benzodiazepines, whereas for subjects undergoing OMT the concurrent abuse of benzodiazepines and amphetamines was more common.
The median number of concurrent drugs was higher among post-mortem cases and untreated drug abusers, indicating the treatments can curb polydrug abuse. Polydrug abuse poses a clear risk of accidental overdose, especially for the drug abusers outside treatment. OMT seemed to have diminished the abuse of buprenorphine and benzodiazepines. Those in treatments also abused less cannabis, opioids, amphetamines, and pregabalin.

P149. **VALIDATION OF CEDIA AND DRI DRUGS OF ABUSE IMMUNOASSAYS FOR URINE SCREENING ON A THERMO SCIENTIFIC INDIKO PLUS ANALYZER**

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Abstinence control for drugs of abuse and ethanol is often demanded for driving licence re-granting during medical and psychological assessment (MPA). For this purpose, a sensitive and reliable screening method for urine samples is needed.
The aim of this study was the validation of Cloned Enzyme Donor Immuno Assays (CEDIA®) and DRI® immunoassays on a Thermo Scientific Indiko Plus analyzer referring to the German driving licence re-granting guidelines. The immunological cut-offs had to be adapted to the following concentrations in urine: THC-COOH: 10 ng/ml (after hydrolysis); morphine (codeine, dihydrocodeine): 25 ng/ml (after hydrolysis); benzoylecgonine: 30 ng/ml; amphetamines (amphetamine, methamphetamine, MDMA, MDA, MDEA): 50 ng/ml; EDDP: 50 ng/ml; benzodiazepines (nordazepam, oxazepam, hydroxy-alprazolam, hydroxy-bromazepam, 7-amino flunitrazepam, lorazepam): 50 ng/ml; ethyl glucuronide: 100 ng/ml.
CEDIA®s were used for THC-COOH, opiates, benzoylecgonine, amphetamine, methamphetamine, EDDP and benzodiazepines. A DRI® assay was used for ecstasy (MDMA, MDA, MDEA) and ethyl glucuronide. Cut-off values and sensitivities were determined using real or diluted urine samples with confirmed concentrations of the analytes in the range of the required MPA cut-offs (confirmation with GC-MS or LC-MS/MS, n = 20 to 43). Due to the low number of real samples containing methamphetamine, MDMA, MDA or MDE, cut-off values and sensitivity of the respective tests were estimated using diluted or spiked samples. The specificity was estimated using confirmed negative urine samples. Within-run precision was determined with low, middle and high concentration quality controls (n = 20).
Cut-off values (immunoassay units) and respective sensitivity: THC-COOH 7 (91%); opiates (morphine) 16 (100%); benzoylecgonine 32 (100%); amphetamine 20 (95%); methamphetamine 20 (100%), ecstasy 34 (100%), EDDP 56 (93%); benzodiazepines 48 (90%); ethyl glucuronide 102 (95%). Specificity: THC-COOH 96%, morphine 100%, ben-

zoylecgonine 100%, amphetamine 97%, methamphetamine 87%, ecstasy 95%, EDDP 88%, benzodiazepines 100%, ethyl glucuronide 100%. Other cut-off values were also evaluated. Within-run precision (CV-%) was below 7.6% for all analytes. There were no false-negative samples for the opiate, cocaine, methamphetamine and ecstasy assay at the above-mentioned cut-offs, and below 10% for all other assays.
The CEDIA® and DRI® assays on the Thermo Scientific Indiko Plus analyzer show sufficient sensitivity with acceptable specificity and precision for drugs of abuse screening in urine and meet the German MPA requirements for the tested analytes.

P150. ANALYSIS OF N,A-DIETHYLPHENETHYLAMINE IN A PRE-WORKOUT SUPPLEMENT AND ITS BEHAVIORAL NEUROCHEMICAL EFFECTS IN RATS

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Pre-workout supplements often contain stimulants for the users not to be exhausted during workout. In this study, it was found that a branded pre-workout supplement manufactured in the United States contains N,α-diethylphenethylamine (NADEP), which was not listed in the ingredients labeling. It was also found in the crystalline powder involved with a previous drug trafficking case. NADEP is controlled in some countries as an amphetamine analog. Its use should be regulated for possible psychoactivity and unexpected toxicity, and toxicological study is required for the safety of public health.
We identified and quantified NADEP in the seized powder and dietary supplement by gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) analysis, and its psychostimulating effects were compared to those of methamphetamine (MA) in experiments on behavioral activities and neurochemical effects of rats.
Structure elucidation of NADEP was performed by NMR analysis. Purity of the seized powder was calculated by the ratio of peak intensities of 1H-NMR spectra of NADEP and maleic acid, and it was used as a standard material after the purity assay. NADEP contents in the supplements were quantitated by a GC-MS method that showed linear standard calibration curve ($R^2 > 0.99$) within the test range, and precision and accuracy values were below 10 %. Behavioral activities of rats were tested using the stereotypy score suggested by Naylor and Olley in 1972. Concentrations of neurotransmitters dopamine (DA), serotonin (5-HT), and their metabolites such as dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in brain tissues were quantitated after administration of NADEP and MA in rats using an LC-MS-MS method validated for this study. Limits of detection (LODs) were 0.3 ng/g for DA and 5-HT and 0.6 ng/g for DOPAC and HVA, respectively. Other validation results such as linearity, precision, accuracy and matrix effect were within acceptable criteria.
The NADEP contents in two types of dietary supplements were 0.40 and 0.44 %, respectively. It means that typical servings of the supplements would be equivalent to about 23 mg of NADEP. Increase of stereotypy score was observed in the NADEP treated rats, but it was less than that after treatment with MA. NADEP administration (5 and 10 mg/kg) resulted in significant increase of DA and 5-HT and decrease of DOPAC and HVA in the striatum tissues which were collected 1 h after administration, though less than those after treatment with MA (5 mg/kg). However, neurochemical levels were soon recovered within 6 h after NADEP administration.
Results of this study suggest that NADEP has significant psycho-stimulatory effects, though the effects were less than those of MA. Thus, NADEP should be carefully monitored to avoid abuse as a psychoactive drug. Because NADEP is not controlled in most countries, this compound should be considered subject to the Medicines Act, and deserves regulation with swift enforcement action.

P151. RAPID SCREENING OF ALPHA-PYRROLIDINOPHENONES IN URINE BY HS-SPME-GC-MS METHOD

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α-Pyrrolidinophenones are a new class of synthetic cathinones that have pyrrolidinyl moiety. They are used alone or mixed with other new psychoactive substances in herbal incenses or research chemicals. Abuse of the α-pyrrolidinophenones has increased recently, and it may cause various problems including acute poisoning, suicide, murder, and traffic accident. α-Pyrrolidinophenones are metabolized mainly to hydroxylated forms, but it has been reported that considerable amounts of unchanged forms are excreted in urine. Matsuta et al reported that concentration of α-pyrrolidinobutiophenone (α-PBP) of 11 abusers' urine samples ranged 485-31,600 ng/mL, and in other report by Shima et al, α-pyrrolidinovalerophenone (α-PVP) concentration of 19 abusers' urine samples ranged 7-32,600 ng/mL. Referring to the reports, most of the urine samples contained over 100 ng/mL of α-PBP or α-PVP.
Due to their pyrrolidinyl moiety, most of the α-pyrrolidinophenones are highly volatile compared to other synthetic cathinones. Head space-solid phase microextraction (HS-SPME) method is limited for low reproducibility, but it shows good sensitivity for volatile compounds. Its sample preparation process is simple, and when coupled with gas chromatography-mass spectrometry (GC-MS), it can be used for simple and cost effective monitoring of the new compounds in biological specimens. Thus, we developed HS-SPME-GC-MS method for rapid screening of α-pyrrolidinophenones in urine samples.
Ten of the α-pyrrolidinophenones including thiophenyl, 4'-methyl, 4'-methoxy and 3',4'-methylenedioxy derivatives were used for this study. Sample amount was set as 200 µL, and it was added to an SPME vial with 40 µL of 5N-NaOH and 100 µL of internal standard solution (0.5 µg of α-PVP-d8 and 2 µg of 3',4'-methylenedioxypyrovalerone-d8 (MDPV-d8) in 1 mL deionized water). Three semi-volatile SPME fibers and 9 sets of extraction conditions were compared

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for efficient extraction of the target compounds. Resultantly, SPME fiber coated with polydimethylsiloxane/divinylbenzene (PDMS/DVB) was selected for highest extraction efficiency, and extraction time of 20 min at 90°C after incubation for 5 min was applied for this study. GC-MS analysis was performed by an Agilent 5975C mass selective detector equipped with a 7890A gas chromatograph, a Gerstel MPS-2 automatic sampler and an HP-5MS column.
Ten of the α-pyrrolidinophenones showed limits of detection (LODs) ranging 5-15 ng/mL, and they were considered sufficient for the analysis of α-pyrrolidinophenones in urine samples. Standard calibration curves of the 10 compounds were linear from limits of quantitation (LOQs) to 4,000 ng/mL, though α-pyrrolidinohexanophenone and 4'-methoxy-α-PVP showed relatively poor linearity with correlation coefficient (R^2) of 0.98. Other validation results such as precision, accuracy and matrix effect were within acceptable criteria.
Though the HS-SPME-GC-MS method is limited for analysis of metabolites and analogs with polar moieties, it showed sufficient extraction efficiency for the analysis of unchanged forms of α-pyrrolidinophenones and their derivatives in urine samples. Its extraction process is so simple that it will contribute to rapid screening of the α-pyrrolidinophenones not only in urine, but in various types of biological specimens.

P152. HEMOPYRROLE (HYDROXYHEMOPYRROLIN-2'ONE, HPL) URINE LEVEL IN PERPETRATORS OF EXTREME VIOLENT ACTS DIAGNOSED WITH PSYCHOSIS

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Hemopyrrole is an abnormal side product of porphyrin biosynthetic pathway, which may have an devastating impact on behavior. Link between aggressive behavior and increased HPL level was postulated in the 60's. Further researches concerning HPL brought contrary results and did not clarify its function and possible role in pathogenesis of aggression.
In our research we hypothesize that heightened level of HPL may correspond with extreme aggressive behavior in subjects diagnosed with psychosis.
LC-MS analysis of HPL level in urine samples, collected from 36 male subjects aged 24 - 63 (mean 39.8, SD 11) diagnosed with mental illness who presented extreme aggressive behavior were performed. Subjects were placed in compulsory psychiatric treatment in hospital with maximal level of security. Control group composed of 22 male subjects aged 23 - 69 (mean 39.7, SD 13) matched with age. An Agilent Technologies HP-1100 Series liquid-chromatograph mass spectrometer (LC-MS) and Waters/Micromass Quattro Micro (LC-MS/MS) were used for the analysis. An atmospheric pressure electrospray ionisation (ESI) was applied. The ions were monitored in SIM mode at programmed fragmentor voltage in CID zone m/z 156, 138, 94 for HPL (m/z of 156 for quantitation) and m/z 340 for papaverine as an internal standard in LC-MS system and in MRM mode with transitions 156→138, 138→123, 138→110 (only for verification) in LC-MS/MS system.
Matrix effect (ME) was calculated from three urine samples containing only a trace amounts of hemopyrrole (<20 ng/mL) that were spiked with analyte to achieve 500 ng/mL. ME calculated value was 96%. Precision and accuracy was determined for three levels 20, 100 and 500 ng/mL each in 5 replicates analysis of spiked urine. Calculated RSD for precision were respectively 4%, 3%, 1 %. Accuracy (n=5, alfa=0.05) were 20%, 11%, 4%. LOD and LOQ (lowest level from calibration curve) were 10 and 20 ng/mL. Correlation coefficient was R^2 =0.993. For freeze-thaw cycle testing urine spiked with hemopyrrole to 500 ng/ml was freezed three times and after thawing at different days concentration was determined. No observable decrease in concentration was noticed. In order to verify the normality of investigated variables statistical tests were carried out. All of them showed that the distribution of hemopyrrole differs significantly from the normal distribution (K-S d=0.22826, p<0.01; Lilliefors p<0.01; Shapiro-Wilk W=0.72303, p=0.00000). Similarly, the distribution of a variable defined as the ratio of hemopyrrole to creatinine differs from the normal distribution (K-S d=0.23043, p<0.01; Lillefors p<0.01; Shapiro-Wilk W=0.73821, p=0.00000). Mean HPL/creatinine ratio in urine in tested group was 95 ng/mg and in the control group was 47 ng/mg.
The hemopyrrole/creatinine ratio differs significantly between the study group and the control group. Further studies are needed to evaluate reasons of HPL elevation and its clinical implications.

P153. DETERMINATION OF ALPHA-PYRROLIDINOVALEROPHENONE (ALPHA-PVP) IN BIOLOGICAL MATERIAL

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α-PVP is a cathinone and pyrovalerone derivative that was synthesized in the 60s of the 20th century. It appeared the drug market in United States in 2011 and in Japan at the end of 2012. In Poland, this synthetic cathinone has become one of the most popular new drugs detected in the components of the so-called 'legal highs' in 2014. Like other cathinones, α-PVP exhibits stimulant effect on the central nervous system. α-PVP action is not well known, it is thought that this substance acts most similar to methylenedioxypyrovalerone (MDPV). Still little is known about the α-PVP toxicokinetics. The literature describes several fatalities, where α-PVP concentrations in the blood were in the range of 411-900 ng/mL. A case of driving under the influence of Ð-PVP has also been described, where the blood concentration was 63 ng/mL.
The aim of the study is to present the α-PVP concentrations observed in biological material of 27 persons using this substance. Presented data is based on the results obtained from the routine expert work carried out at the Institute of Forensic Research (IFR) in Krakow in 2014-2015.
The biological material (blood and urine, as well as vitreous humor and tissues in fatal cases) were collected mainly from drivers stopped for routine control (10 drivers + 1 passenger), 3 individuals involved in road accidents,

4 subjects suspected of drug possession, 3 people who have been charged for threatening and/or violence, 1 person suspected of theft, as well as 5 individuals intoxicated with this substance (including 4 fatalities). The samples (0.2 mL) were extracted with n-butyl chloride. The analysis for α-PVP was performed using liquid chromatography coupled with mass spectrometry (LC-MS). Separation of the analytes was achieved on a Zorbax SB-C18 column (2.1x50 mm). The detector was working in multiple reaction monitoring mode (MRM). The precursor and three fragment ions for α-PVP, were: 232.2→126.1, 232.2→91.0, 232.2→77.0. Positive electrospray ionization (ESI) was applied.
α-PVP was the only drug present in the investigated materials in twelve cases (including one fatality). In other cases were present along with the α-PVP: amphetamine, benzoylecgonine, THC, other new drugs (3-MMC, MDPBP, pentedrone), medicinal drugs and ethyl alcohol. In the blood of drivers (including those who were injured) the concentrations of α-PVP ranged from 5 to 94 ng/mL. In one of the four fatalities the concentrations of this cathinone derivatives was 650 ng/mL in blood, and 3850 ng/mL in urine, however the ethyl alcohol was also determined at concentrations of 3.12 mg/mL and 4.61 mg/mL, respectively. In two cases, the α-PVP concentrations in blood and urine were lower - 101 and 86 ng/mL; 118 and 35 ng/mL, but the presence of other substances in blood was also revealed.
Performed studies indicate that α-PVP is now one of the more popular cathinones on the drug market. It is often taken together with other drugs, in particular 3-MMC. The obtained results correlate to a few cases described in the literature. The aforementioned cases are the most extensive collection, where the α-PVP concentrations were determined in biological material.

P154. ATM4G – AN ADDITIONAL MARKER FOR THE INTAKE OF STREET HEROIN

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Heroin is rapidly deacetylated into 6-Monoacetylmorphine (6-MAM) and morphine. Papaverine, noscapine and 6-acetylcodeine (6-AC) are considered to be impurities of street heroin. Primarily the acetylated compounds 6-MAM and 6-AC can help to distinguish heroin intake from other reasons of morphine findings (i.e. the "poppy seed defense"). The opium alkaloid thebaine is also acetylated and the molecule is afterwards rearranged, conjugated with glucuronic acid and excreted into the urine (ATM4G, acetyl-thebaine-metabolite-4-glucuronide). This molecule could also be a potential marker of heroin consumption.
The aim of the study is to verify if ATM4G can be used as a new potential heroin marker in urine samples.
ATM4G was synthesized after a published protocol [[Chen et al., Drug testing and analysis 6.3 (2014): 194-201].
Urine of 19 patients, who presumably consumed heroin, were measured for the above mentioned analytes by liquid chromatography-mass spectrometry (separation with a Restek Allure PFPPropyl column®) after protein precipitation (for ATM4G) or a liquid liquid extraction with n-chlorobutane/acetonitrile (80:20; for the other opioids). Limits of detection were 50 ng/ml for ATM4G, 0.33 ng/ml for acetylcodeine, 0.22 ng/ml for noscapine, 0.05 ng/ml for papaverine and 1.4 ng/ml for 6-MAM.
In seven cases - in addition to morphine and codeine - noscapine and ATM4G could be detected in urine. 6-MAM (5 of 7), papaverine (6 of 7) and acetylated codeine (5 of 7) could not be detected in all seven cases. In the remaining twelve urine samples, which were not positiv for ATM4G, papaverine (1 of 12), noscapine (2 of 12) and codeine (7 of 12) but no 6-MAM or 6-AC could be detected.
Since noscapine, papaverine and codeine findings can also be explained by the intake of poppy seed products or special medications, these substances have not yet been proven to be safe heroin markers. Due to the more reliable detection of ATM4G compared to 6-MAM and 6-AC, ATM4G can be used as an additional marker for heroin consumption. Additionally, ATM4G seems to be excreted into urine with a broader detection window than other markers and should be measured in cases when heroin consumption has to be distinguished from poppy seed intake. However, excretion studies after the intake of heroin and poppy seed products, respectively, must be done. Results of an ongoing excretion study after the consumption of different poppy seed products are currently being evaluated.

P155. SIMULTANEOUS ANALYSIS OF AMPHETAMINES, KETAMINE AND ITS METABOLITES IN URINE BY AUTOMATED DPX-LC-MS/MS SYSTEM

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Analysis of controlled substances in biological sample always requires multiple sample preparations steps followed by chromatographic instrumental analysis. Routinely, the laboratory uses an off-line solid phase extraction (SPE) for sample preparation followed by gas chromatography-mass spectrometry (GC-MS) for the analysis of amphetamines and ketamine in urine, which is time-consuming and laborious. Typically, a batch of 30 urine samples requires approximately 20 hours of sample processing and analysis time. Hence, to improve the throughput of the analysis, the development of an online automated system is deemed necessary for routine testing.
To develop a fully automated system for simultaneous analysis of amphetamines, ketamine and its metabolite by using Gerstel® MultiPurpose Sampler (MPS) coupled with Agilent 1290 UPLC-ABSciex® QTrap 4500 tandem quadrupole mass spectrometer. This will improve the throughput of the routine testing for controlled substances in urine in the laboratory.
Disposable Pipette Extraction (DPX) was employed for the automated extraction. 250 µl of a urine sample was extracted using DPX which contained loosely packed reversed phase salting (DPX-RP-S) sorbent in a disposal

pipette. MPS was used for the automated sample extraction, reconstitution and injection. The injector of MPS is equipped with a Dynamic-Load Wash (DLW) module to reduce carryover for injection to the ABSciex® QTrap 4500 tandem quadrupole mass spectrometer coupled to an Agilent 1290 UPLC for analysis. The analytes studied were amphetamines (including methamphetamine, amphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyethylamphetamine (MDEA), phentermine, fenfluramine, ephedrine and pseudoephedrine), ketamine and norketamine. Chromatographic separation was achieved on a Phenomenex Kinetex® XB-C18 (2.1mm x 150mm, 2.6µm) column using a 0.1% formic acid in ammonium formate (10 mM)/acetonitrile gradient.
The method was validated according to the SWGTOX Standard Practices for Method Validation in Forensic Toxicology for quantitative analysis using fortified urine samples. Linearity was achieved between concentration range of 100 ng/ml and 1,000 ng/ml for all analytes with the regression coefficients ranged from 0.9213 to 0.9943. The limit of detection (LOD) and limit of quantitation (LOQ) were found to be at 50 ng/ml and 100 ng/ml, respectively, based on 3 MRM transitions. The intra-day and inter-day precision were found to have coefficients of variation (CVs) ranged from 2.6% to 8.9%, and their accuracy ranged from -6.8% to 6.7%. No significant interference from other common controlled drugs was observed from the study. Ion suppression or enhancement was measured to be within -6.6% to 7.7%. No carry over was observed in the urine blank samples for concentration up to 10,000 ng/ml. The extraction recoveries for all analytes ranged from 79.9% to 127.1% for concentrations of 150, 400 and 800 ng/ml. A parallel study with GC-MS analysis was performed using 100 routine urine samples and the results obtained were found to be within ± 20% from the mean concentrations.
The simultaneous analysis of amphetamines, ketamine and its metabolites in urine was successfully developed and validated on an automated DPX-LC-MS/MS system. This method has greatly improved the workflow for routine urine testing with saving on the manpower and instrument time.

P156. QUANTITATIVE AND QUALITATIVE ANALYSIS OF SYNTHETIC CANNABINOIDS DETECTED IN 'HERBAL HIGHS'

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'Herbal highs' is a name used commonly for products containing herbal material laced with synthetic cannabinoids (SC). Their activity is connected with acting on the cannabinoid receptors in the human body similar to delta-9-THC, the major component of cannabis plants. In 2014, 30 new SC were detected for the first time on the European market, bringing the total number reported to the EU Early Warning System to 134 substances.
The paper is a 3-years retrospective study aimed at detailed analysis of qualitative and quantitative composition of psychoactive components of 'herbal highs' seized by law enforcement over the last three years (2012 - 2014)in Poland.
In total, 1148 'herbal highs' differing in names and package sizes were analysed in the aforementioned period. Sample preparation procedure covered homogenization, weighting and extraction with methanol. The extract was centrifuged and diluted with an appropriate solvent, followed by addition of internal standards. GC/MS analyses were performed on an HP-5MS capillary column, and acquisition was carried out in the scan mode (m/z 29 - 600 amu). Chromatographic separation in UHPLC/UVvis method was realized on Kinetex C18 column with a mobile phase gradient. The UVvis spectra were recorded in a range of 200 to 400 nm.
27 SC were identified in the preparations over the last 3 years. UR-144 was definitely the most popular SC and it was detected in 63% of the products. The other common cannabinoids were: RCS-4 (13%), XLR-11 (8%), 5F-AKB48 (7%), JWH-081 (6%) and JWH-122 (4%). In contrast to 'herbal highs' seized in previous years (2009 - 2011), most samples contained only one active ingredient. Only 15% of the products were mixtures and the most popular were composed from the substances with similar chemical structures: UR-144 + XLR-11 (7%) and JWH-122 + RCS-4 (2%). Net mass of the seized materials was usually below 1.0 g (83% of all analysed samples), and typically was in the range 0.4 - 0.6 g. The doses of the most popular cannabinoids contained in individual portions/packages up to the net mass of 1.0 g were determined. The content of UR-144 was in range 2 - 200 mg (80 mg in average in products in which UR-144 was the sole active component), RCS-4 from 0.3 to 65 mg (20 mg), XLR-11 from 0.3 to 13 mg (6 mg). Besides cannabinoids, other new psychoactive substances were detected in herbal blends, and α-PVP was the most popular (3% of the products).
The study confirmed high diversity of the content of 'herbal highs'. Unpredictable, inconsistent qualitative and quantitative composition of the products is the most dangerous threat connected with abuse of SC and may pose a serious health problems to the users. Many products contained controlled drugs (RCS-4, JWH-081, JWH-122), which could have serious consequences as the possession of such substances is punishable in Poland by 2 years in prison.

P157. DETECTION OF DRUGS IN BIOLOGICAL SPECIMENS FROM EMERGENCY ROOM PATIENTS

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Because there is no systematic toxicological analysis was developed in Korean hospitals yet, usually intoxicated patients were treated by their symptoms not by what they administered. Therefore it is urgent to establish the detection method to identify the drugs and chemicals for the treatment. For this purpose, 28 intoxicated patients delivered from 9 Feb to 30 Mar for medical treatment at Chungnam national university hospital emergency center were studied.
Firstly the detection method for drugs and chemicals in blood and urine from Emergency Room (ER) patients was studied by GC/MS using SPE extraction. Secondly, because Doxylamine and zolpidem which are commonly encountered at ER room, the blood concentration and the clinical symptoms of these were compared to see the correlation between the level of blood and symptoms.
28 blood and 24 urine specimens were collected from the 28 patients who delivered by Paramedic to Chungnam national University Hospital. After the comparison of liquid-liquid extraction (LLE) and solid-phase extraction (SPE) for extraction efficiency, specimens were extracted with SPE and analyzed using gas chromatography-mass spectrometry. For method validation, linearity, limit of detection (LOD), limit of quantitation (LOQ), intra- and inter-day precision, and accuracy were studied. The blood concentrations of doxylamine in two cases and zolpidem in three cases were determined by this method. Clinical symptoms and GCS (Glasgow coma Score) were measured at ER. GCS ranged from 3 (full coma state) to 15 (full alert)
The SPE proved more accurate and precise compared to LLE. Zolpidem and Doxymaine were validated. Among the 28 blood and 24 urine specimens, Tramadol was detected in two, zolpidem in three, doxylamine in two, imipramine in two specimens. Pesticides, herbicides and chemicals were detected in other specimens. Clinical symptom in one patient whose blood level of doxylamine was 37ng/ml included mental change (drowsy mental state) and GCS was 9 (eye 2, verbal 5, motor 5) while in the case of blood level was lower than detection limit, the GCS was 15. The blood levels in Zolpidem cases ranged from 200 to 900 ng/ml and drowsy mental state was shown in all three cases. GCS ranged from 7 (eye 1, verbal 1, motor 5) to 14 (eye 4 verbal 4 motor 5).
The detection of drugs and chemicals in blood and urine was developed and specimens from 28 patients from Emergency Room (ER) patients were analyzed by this method. The blood level of doxylamine and zolpidem were well correlated with the clinical symptoms of mental change and the score of GCS. It will be a useful information to clinical toxicologists.

P158. NEW PSYCHOACTIVE SUBSTANCES AND THEIR DETERMINATION IN BIOLOGICAL MATRICES

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New psychoactive substances (NPS) have become a global phenomenon mainly because of on-line distribution, which has a significant impact on their spreading. Recently, methoxetamine (MXE) belonging to this family has been noted by the EU Early Warning System (EWS). There are warning numbers of 110 intoxications and 20 deaths associated with this dissociative anaesthetic possessing similar effects as ketamine.
The aim of this study was to develop a method suitable for the sample preparation and MXE and its main metabolites determination in urine, serum and several tissues (brain, lungs, and liver). Predicted MXE metabolites and one deuterated MXE analogue were synthesized in our laboratory for the confirmatory purpose.
Following approaches were applied to prepare samples: a) plain dilution (100 µL of urine); b) precipitation of proteins with acetonitrile (50 µL of serum); and c) homogenization with SALLE extraction (100 mg of tissue). LC-MS determination of the analytes was based on the application of Zorbax Eclipse Plus C18 column (50 × 2.1 mm i.d., 5 µm) hyphenated with a triple quad MS system 3200 API in positive electrospray mode. We used MRM mode with two transitions.
The developed LC-MS method of quantification of MXE, normethoxetamine, O-desmethylmethoxetamine and their hydroxylated and reduced forms was validated for each type of matrix. The matrix-matched calibrations were prepared with regression coefficients 0.996-0.999. LODs (3 x S/N) and LOQs (10 x S/N) were estimated as <0.5-2.5 ng/mL and 0.5-5.0 ng/mL, respectively, except of dihydro-O-desmethyl-normethoxetamine and dihydro-normethoxetamine, which are less common. Blank matrices were spiked on two different levels (20 and 200 ng/mL; 10 and 100 ng/mL) to evaluate bias (51-146%) and repeatability (1-20%). Selectivity was evaluated by the analysis of 6-8 analyte-free samples. Carry-over (<0%) and matrix effects (-9-46%) were also determined. The validated procedure was applied to real samples collected in toxicological trials on rats.
We confirmed and quantified metabolites predicted in literature based on the synthesized standards.

Poster abstracts

P159. AN EVALUATION OF THE USE OF IMMUNOCHEMICAL ASSAYS FOR THE SCREENING OF SYNTHETIC CANNABINOIDS IN URINE

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Synthetic cannabinoids are constituents of herbal smoking preparations, sold over the Internet by brand names such as Spice and K2, which are used as substitutes for cannabis. In a clinical setting special methods targeting the metabolites of synthetic cannabinoids are required.
The aim of this study is to evaluate the screening procedures for the detection of synthetic cannabinoids in urine.
In this study a homogenous enzyme immunoassay, the K2 (Synthetic Cannabinoids-1) HEIA kit, from Immunalysis Corporation was used for urinary screening analysis of synthetic cannabinoids and metabolites. The method was calibrated with JWH-018-N-pentanoic acid using a cutoff level at 5 ng/mL. Confirmation was performed with an LC-MS/MS confirmation method after enzymatic hydrolysis and liquid-liquid extraction. The method allowed detection of all 31 commercially available metabolites, from 21 different synthetic cannabinoids, using a cutoff level of 1 ng/mL for each substance. During a two years period (Mar. 2013 - Feb. 2015) 42179 clinical urine samples were analyzed with the screening method and 1879 (4.5%) were found positive. During the same period, 987 samples, testing positive in the screening, were subject for the LC-MS/MS confirmation procedure. In 219 samples (22%) the presence of cannabinoid metabolites was confirmed.
During a two years period 42179 urine samples were analyzed with the screening method and 1879 (4.5%) were found positive. During the same period, 987 samples, testing positive in the screening, were subject for the LC-MS/MS confirmation procedure. In 219 samples (22%) the presence of cannabinoid metabolites was confirmed. The monthly rate of positive results in the screening assay varied over time between 2 and 11%. The rate of positive confirmation of the positive screening results decreases from 88% to below 10% over the study period. In addition, a complementary homogenous enzyme immunoassay, called the K2 (Synthetic Cannabinoids-2) HEIA kit, from Immunalysis Corporation, for urinary screening analysis of synthetic cannabinoids and metabolites was evaluated. The method is calibrated with UR-144-pentanoic acid and has a proposed cutoff level at 10 ng/mL. The within-assay coefficient of variation (%CV) at 5, 10 and 15 ng/mL were 12.8, 3.2 and 5.8 respectively and the day-to-day %CV at at the same levels, were all below 4.9. 725 urine samples were analyzed with this method together with the Cannabinoids-1 screening assay. Using a cutoff at 5 ng/mL for both assays, 7 samples were found positive with both assays, 26 were positive with only Cannabinoids-1. 5 samples were positive with Cannabinoids-2 only and in none of them the presence of cannabinoid metabolites could be confirmed.
Since the crossreactivity in the two immunochemical assays is not fully investigated and since the LC-MS/MS requires more commercially available reference materials, all the tests has a limited suitability in a clinical setting.

P160. AN LC-MS/MS CONFIRMATION METHOD APPLIED IN ROUTINE ORAL FLUID DRUG TESTING USING SIMPLE SAMPLE PREPARATION

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The sample collection of oral fluid (OF) offers some advantages over more traditional matrices, such as urine and plasma. The collection is, e.g., easily performed and considered less invasive. OF has therefore become applied in workplace drug testing programs.
The aim was to develop a fast and reliable LC-MS/MS method for the confirmation analysis of some drugs of abuse in OF, collected with the QuantisalTM OF collection device. The method needed to be robust and involve a simple sample preparation. The method was developed for amphetamine, methamphetamine, cocaine, benzoylecgonine, morphine, codeine, 6-acetylmorphine, diazepam, oxazepam and Δ9 tetrahydrocannabinol (THC).
The samples are collected with the QuantisalTM oral fluid collection device and diluted in buffer (1+3, v/v). The mixtures are shipped to the Department of Clinical Pharmacology for screening by immunoassay (Beckman Coulter, Olympus AU640/AU680 and Oral Fluid HEIA Kits, Immunalysis). Positive findings are confirmed by the developed LC-MS/MS method. The samples are centrifuged and deuterated internal standards are added to 100 µL of the samples. After precipitation of proteins, with organic solvents, and dilution with the mobile phase, a volume of 2 µL is injected into the LC MS/MS system (Dionex Ultima 3000 UHPLC coupled to TSQ Vantage, Thermo Scientific). The separation is carried out by the use of an Acquity UPLC BEH Phenyl column (2.1 × 100 mm, 1.7 µm, Waters). The run time is 4.0 minutes per injection. The method was fully validated. The range was determined to 4 (LLOQ) Ð 400 ng mL-1 for all compounds except THC, for which the range was 2 (LLOQ) - 200 ng mL-1. The accuracy, determined at three concentrations, was between 94.2 and 104.2 %. The total imprecision (CV%) was ≤ 10.8 %. The matrix effects were in the range 86.3 - 115.6 % for all compounds except for benzoylecgonine. For benzoylecgonine the matrix effect was 23.2 %.
In this study, we show that the volume of the collected OF using QuantisalTM is in the range 0.70 – 1.68 mL (n = 100) and that the collection pad in the collection device can be discarded upon arrival at the laboratory without alteration of the sample concentrations in the buffer solution. During a period of 13 months, a total of 16 290 samples from workplace drug testing programs were screened by immunoassay for the various classes of drugs. A total of 203 confirmations were performed by the LC-MS/MS method. THC was the most frequently found compound, 82 samples were screened with a positive result for THC, and out of these samples, 56 samples were confirmed.

A reliable LC-MS/MS method was developed for the determination of some drugs of abuse in oral fluid. To simplify the sample preparation, it was showed that the collection pad could be removed from the QuantisalTM collection device. Furthermore, the "dilute and shoot" approach was found to be successful for the subsequent LC-MS/MS analysis. The high sensitivity of the method makes it suitable not only for confirmation, but also for screening purposes. The method will be further developed for additional compounds.

P161. SIDE EFFECTS AND TRENDS IN THE CONSUMPTION OF ANABOLIC-ANDROGENIC STEROIDS (AASS) AND PSYCHOTROPIC DRUGS IN BODYBUILDERS

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A large number of adolescents and young adults abuse AASs to improve their physical fitness and appearance. This abuse involves more than a desire to enhance the user's appearance or sporting performance: it appears to have much in common with the use of substances that induce drug dependence. Persons who abuse AASs also tend to abuse other psychotropic drugs (PD). This trend is alarming, and a phenomenon that may contribute to the serious side effects of AAS use, such as the negative reactions in the liver and several cardiovascular risk factors.
To investigate the trends in the consumption of AASs and PD and their physiological side effects in competitors and amateurs bodybuilders.
A total of 20 volunteers were selected whose were competitors and amateurs bodybuilders, and that had affirmed the consumption of AASs and PD. The survey methodology involved three stages, which were applied to all volunteers'. 1) Administration of a self-completion questionnaire. 2) Assessment of biochemical parameters of volunteers' lipoprotein fractions, triglyceride levels and hepatic enzymes. 3) Toxicological urinalysis of the studied PD. Urine samples were processed by a Liquid Liquid Extraction (LLE) procedure, which were followed by two steps (basic and acid condition). Basic hydrolysis was performed using NaOH and ethyl acetate. Acetic acid was added to the supernatant and the remaining sample to adjust pH 4. Mixed solution of n-hexane: ethyl acetate was successively added to the sample. Extracts were derivatized with MSTFA then injected into a GC/MS.
1) A total of 20 questionnaires were collected from volunteers', which affirmed the consumption of AASs and PD. The substances examined are mainly used to improve physical appearance and performance and PD. 2) Average levels of total and free testosterone of the all participants showed an increase of 20 to 60% compared to reference values. Analysis of volunteers' results of biochemical parameters were performed on an auto scaled data Principal Component Analysis. Results were based on 73% of the total number of components. The samples showed significant effects in blood lipid levels and hepatic enzymes with a reduction in HDL levels and increased in hepatic enzymes (GOT, GPT) and total Cholesterol in the AASs, cocaine, amphetamine and MDMA group users. 3) LLE method in urine presented extraction efficiencies, good recovery and selectivity to analyze low concentrations of the proposed analytes (COOHTHC, amphetamine, benzoylecgonine, methamphetamine and MDMA). Results of urinalysis confirmed the self-reports in questionnaires.
This study showed that the amphetamine, MDMA and cocaine are drugs most used among these group of 20 volunteers after cannabis, because that may induce then a sensation of an "energetic explosion" that enables to train harder. Given the potential biological adverse effects of abuse at high doses AASs and PD, the consequences for these users and their immediate surroundings, the importance of prevention is obvious. If the reductions in their HDL levels were maintained it could result an increased risk of cardiovascular disease and can be fatal. In addition these increase in hepatic enzymes activity with prolonged abuse could adversely affect other aspects of liver structure and function.

P162. A NON-FATAL SELF-POISONING ATTEMPT WITH SILDENAFIL

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The phosphodiesterase type 5 inhibitor sildenafil is used for the treatment of pulmonary hypertension and erectile dysfunction. Although sildenafil is not generally known for its use as a self-poisoning drug, reports of intoxication cases have been described including some with a lethal outcome. The presented case is of a 56-year-old man who claimed to have undertaken an unsuccessful suicide attempt, by ingestion of 65 tablets of 100 mg sildenafil. He arrived in the emergency room with severe vomiting and symptoms of blurred vision. Of note was a sinus tachycardia of 100 bpm, but no signs of hypotension or priapism.
To develop a chromatographic method for the investigation of the sildenafil intoxication claim and for the pharmacokinetic monitoring of serum sildenafil concentrations.
In order to quantify the sildenafil level in serum, a high performance liquid chromatography - photodiode array method was developed and validated according to European Medicine Agency guidelines. A liquid-liquid extraction (hexane: ethyl acetate, 7:3) was applied to 350 µL serum after addition of the internal standard (prazepam) and pH-adjustement. After evaporation of the organic layer, the extract was reconstituted in 70 µL water: methanol (3:1). Twenty microliter was injected on the column (Agilent ZORBAX Eclipse Plus C8, 3.0 x 150 mm, 3.5 µm particle size). A gradient elution was applied (solvent A: 10 mM phosphate buffer, pH 2.3; solvent B: 10 mM phosphate buffer pH 2.3: acetonitrile, 2:8) and quantification was performed at 225 nm. The limit of detection was 0.008 µg/mL and the method showed linearity between 0.025 and 2.5 µg/mL. Intra- and inter-day variation at lower limit of

quantification/low/mid/high concentrations (0.025/0.075/0.250/2.000 µg/ml) were always below 10.2 and 12.9%, respectively. Inter-day accuracy at the same levels ranged between -5.8 and 0.4%. Recovery rates were 79 ± 8% for the low quality control level (0.075 µg/mL) and 80 ± 6% for the high level (2 µg/mL). Tenfold dilution integrity also met the requirements (97 ± 10%).
The diagnosis of sildenafil intoxication was confirmed by analysis of multiple serum specimens of the patient during 3 days in which the drug clearance could also be studied. The patient had a serum concentration of 22.2 µg/mL sildenafil at the time of presentation at the emergency department, which is far above the therapeutic peak concentration of 0.5 µg/mL after a single oral dose of 100 mg. The serum concentration of this patient further declined to 9.2 and 2.3 µg/mL, respectively 5 and 14 hours later, revealing a biological half-life of approximately 4.2 hours, which is in accordance with literature data.
To the best of our knowledge, this patient took the highest sildenafil dose, which resulted in the highest serum concentration level, ever reported. In this subject, sildenafil showed a good tolerability since few symptoms occurred and only moderate supportive therapy was needed for full recovery without sequelae. Nevertheless, the unofficial sale of sildenafil and variants, and the exponential increase in online pharmacies remains a major concern, especially for patients with pre-existing comorbidities or multiple drug intake.

P163. REDOX BALANCE AND DNA INTEGRITY IN SEMEN OF PRIMARY IDIOPATHIC INFERTILE MALE CANNABINOID SMOKERS

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Cannabis smoking has serious impact on sperm DNA integrity ,production and morphofunction. In addition, oxidative stress has long been identified as having influence on males reproductive health.
To assess sperm nuclear DNA reaction and seminal plasma oxidative stress status in cannabinoid smoking primary infertile men.
Semen samples were obtained from 40 cannabinoid smoker married men and 40 strict non cannabis smoker (Tobacco smokers) married men, half of each group was primary infertile. The seminal samples of both groups were free from leukocyte and erythrocyte . After liquefaction, semen samples were analyzed for: (i) semen parameters by a computer assisted semen analyzer. ii) seminal plasma oxidant-antioxidant status including: the lipid peroxidation (LPO) index [malondialdehyde (MDA)], and some antioxidants [α-tocopherol, vitamin C, superoxide dismutase (SOD) and reduced glutathione]. iii) DNA denaturation state was assessed using FACSort flow cytometer.
Cannabis smoking resulted in significant elevation of mean MDA in seminal plasma than the corresponding non cannabis smokers ($p<0.001$), this finding was more prominent in infertile cases. Enhanced LPO of sperm plasma membranes induced decrease in sperm motility and viability. Whereas, measuring the antioxidants; glutathione, α-tocopherol, vitamin C and SOD concentrations in seminal plasma were significantly reduced in cannabis smokers group than in the corresponding non cannabis smokers group ($P<0.01$) but significantly higher in the infertile cases of both groups. Spermatozoa of the cannabis smokers group showed unfavorable alternation in sperm chromatin quality and even induce its damage.
Cannabis smokers men suffers from unfavorable effect on sperm DNA integrity and seminal quality as well as imperfect scavenging antioxidant activity in seminal plasma. Cannabis smoking is dramatically deteriorate DNA integrity compared with tobacco smoking and consequently male infertility. FACSort flow cytometer is a distinctive determinant of sperm DNA denaturation in primary idiopathic infertile male cannabis smokers.

P164. EVALUATION OF ORBITRAP ULTRAHIGH RESOLUTION MASS SPECTROMETER FOR QUANTITATIVE ANALYSIS OF BARBITURATES IN URINE

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The ultra-high resolution mass spectrometers are used in forensic laboratories screening methods. Implementation of these instruments for confirmation is still under evaluation. Here we investigated how the quantitative performance of an ultra-high resolution Orbitrap mass spectrometer compares to that of a triple quadrupole mass spectrometer.
To develop and evaluate performance of a quantitative method for the analysis of barbiturates in urine on a Thermo Scientific™ Q Exactive™ Focus ultra-high resolution quadrupole-Orbitrap mass spectrometer and to compare the results to those obtained on a Thermo Scientific TSQ Endura™ triple quadrupole mass spectrometer.
The compounds analyzed in this study were amobarbital, butalbital, pentobarbital, phenobarbital and secobarbital. Samples were prepared by addition of internal standard (stable-labeled analogs) to the urine followed by 20-fold dilution with water. The samples were analyzed by gradient HPLC and a hybrid quadrupole-Orbitrap mass spectrometer. Mobile phases were 10 mM ammonium acetate in water and 100% acetonitrile. The column used was an Accucore C18 2.6µm, 50x2.1mm, fused core. The Q Exactive Focus mass spectrometer was equipped with a heated electrospray ionization probe (HESI II) and operated in Parallel Reaction Monitoring (PRM) mode. In PRM mode, the MS isolated each precursor mass with the quadrupole and then collected MS/MS spectra in the Orbitrap with a resolution of 30K for every individual compound. The two most abundant fragments in MS/MS spectrum were used for quantitation and confirmation, and chromatographic peaks were reconstructed from the MS/MS spectra

with a mass accuracy of 5 ppm. Calibrators and controls were prepared in synthetic urine to evaluate limit of quantitation (LOQ), linearity, accuracy and precision. Matrix effects were evaluated by analyzing spiked urine from six different donors and calculating %recovery. Additionally, internal standards signal recovery in 100 donor samples was calculated and compared to average signal in calibrators. Results collected with this Orbitrap instrument were compared to results obtained with triple quadrupole mass spectrometer.
Limits of quantitation were defined as the lowest concentration which had back-calculated values within 20%, ion ratios within defined tolerance, and quality controls meeting the above two requirements. Using these criteria, LOQ ranged from 5-25 ng/mL for the various compounds tested. The upper linearity limit was 2000 ng/mL, the highest concentration analyzed in this evaluation. Method precision was better than 15%. Limited matrix effects were observed and were corrected by deuterated internal standards. Internal standard recoveries in donor samples calculated against signal in calibration standards were within 50-150%. Data collected with ultra-high resolution mass spectrometer correlated very well with data collected with the same method implemented on a triple quadrupole mass spectrometer (R^2=0.96).
We showed that a method for the analysis of barbiturates in urine implemented on Q Exactive Focus ultra-high resolution mass spectrometer met forensic laboratory requirements. Results correlate very well with a method that used a triple quadrupole mass spectrometer. Improved selectivity on the high resolution instrument results in very low to no background in reconstructed chromatograms which significantly improves accuracy of peak integration and allows for rapid data review.

P165. DEVELOPMENT OF AN UHPLC TANDEM MASS SPECTROMETRY MULTI-COMPONENT METHOD FOR DETERMINATION OF NEW DESIGNER BENZODIAZEPINES IN URINE

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The supply of unregulated "new psychoactive substances" (NPS) has shown a steady increase over the past six years, according to data reported to the EU Early Warning System operated by the European Monitoring Center for Drugs and Drug Addictions (EMCDDA) and Europol. In addition to stimulants (e.g. cathinones) and synthetic cannabinoids ("spice") which have dominated the NPS panel, there is currently a growing diversity of psychoactive drug classes. In Sweden, unregulated designer opioids and benzodiazepines recently started to be supplied by web-based vendors and they have reached an increasing popularity among NPS users.
This study was undertaken to develop an ultra high-performance liquid chromatographic-tandem mass spectrometric (UHPLC-MS/MS) multi-component method for qualitative and quantitative determination of some new designer and unregulated benzodiazepines in urine samples. The aim was also to study the presence of these substances among urine samples sent for routine laboratory analysis of drugs of abuse.
The benzodiazepines covered by this study were diclazepam, etizolam, flubromazepam, meclonazepam, phenazepam and pyrazolam. The UHPLC-MS/MS method involved direct sample dilution with internal standard, reversed phase separation on a BEH phenyl column, and MS/MS detection using electrospray in positive mode. Method validation was performed according to the European Medicines Agency guidelines. For the prevalence experiments, surplus volumes of patient samples sent to the laboratory for routine testing of drugs of abuse, and screening positive (CEDIA) for prescription benzodiazepines, were used.
The method had a total runtime of 3.1 minutes and gave quantification limits (LLOQ) in the range 1-20 ng/mL for the investigated benzodiazepines. The detector response was linear within calibrated measuring ranges and the coefficient of variation was below 20% at the LLOQ and below 15% at higher concentrations. No analytical interferences above the accepted value were found for any of the analytes. When the method was applied on patient samples, intake of all studied benzodiazepines except diclazepam could be confirmed. Flubromazepam and pyrazolam were the most common NPS benzodiazepines in the beginning of 2014, while meclonazepam and etizolam were more common in the latter part of the year. Among the samples screening positive for benzodiazepines but confirmed negative with the standard routine confirmation method, about half contained one of the benzodiazepines covered by the study.
These results demonstrated that several new unregulated benzodiazepines are being used in Sweden. This highlights the importance to evaluate not only intake of the standard set of prescription benzodiazepines, but that confirmation methods should be updated to also cover the new "designer" benzodiazepines made available by web-based NPS vendors.

P166. NEUROBEHAVIOURAL EFFECTS OF EXPOSURE TO FLUORIDE IN THE EARLIEST STAGES OF RAT DEVELOPMENT

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It is known that exposure to high concentrations of Fluoride (F) produces deleterious health effects in human population. However, in the last years it has been concluded that low concentrations of F may have adverse health effects as well. Transplacental passage of F and its incorporation into fetal tissues has been demonstrated. Therefore, the purpose of the present work was to study the effects of the exposure to low levels of F during pregnancy and lactation on the central nervous system functionality
The purpose of the present study was to study in young and adult rats, the effect of exposure to low concentrations of F during pregnancy and lactation, on sensory-motor reflexes and on the anxiety level.

Pregnant female rats were exposed to 5 and 10 mg/L F in drinking water during pregnancy and lactation. Starting on postnatal day 3, each pup received a battery of developmental tests: Righting reflex, Cliff aversion and Negative geotaxis. Also, the ear and eye opening were assessed. One test trial per day was given to the pups on each test. The dependent variable analysed for each test consisted in the postnatal day until the following criteria were reached by each pup. In the 45- and 90-day-old offspring, anxiety was determined using an elevated plus maze. The plus maze consisted of two arms enclosed and located perpendicularly to two open arms. The test exploits a rodent's natural conflict between avoidance and exploration of open and elevated areas. The rats were placed in the center of the maze and were allowed to explore the maze freely for 5 min. The following parameters were calculated: i) percentage of time spent in the open arms, ii) percentage of entries in the open arms, and iii) total number of arm entries. Increased time spent and entries in the open arms are consistent with a decrease in anxiety behavior.
A significant delay in the development of eye opening was observed in all offspring whose mothers had been exposed to the two F concentrations tested ($p<0.001$). A low index of anxiety in the young females ($p>0.01$for 5mg/LF and $p<0.05$ for 10 mg/L F) and in all adult offspring ($p<0.001$ for female and male) exposed to the two F concentrations tested was also detected.
The present study shows that exposure to low F concentrations during pregnancy and lactation produces dysfunction in the central nervous system mechanisms which regulate motor and sensitive development and anxiety.

P167. LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LC-MS-MS) DETERMINATION OF DOCETAXEL AND ITS METABOLITS IN BLOOD: A PHARMACOKINETIC STUDY.

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The therapeutic drug monitoring (TDM) is used in clinical practice for optimizing the therapeutic treatment with various drugs. Although the variation of the pharmacokinetic parameters, notoriously, also influences the success of pharmacological treatments with chemotherapeutic agents, the use of TDM in oncology has always been limited due to the fact that the therapeutic index of the most widely used anticancer was not defined. Currently, the chemotherapeutic agents are administered according to the physical characteristics of a patient. The dose is often formulated as mg/m2 of body area or mg / kg of body weight of the patient. Although this dose has already been customized, the clinical efficacy and toxicity of therapy vary greatly from one patient to another. In part, the pharmacodynamic changes observed can be explained on the basis of pharmacokinetic changes (changes in the speed and extent of absorption, distribution, metabolism and elimination).
The aim of the study was to find a possible correlation between patient's gene pattern and the pharmacokinetics of docetaxel (Taxotere®) through the direct determination of these compounds as well as their main metabolites in whole blood (M1-M3, stereoisomers products from cyclization of M2 and M4, product of oxidation of M1-M3).
Whole blood samples containing internal standard (Paclitaxel at the concentration of 1000 ng/ml) were acidified with acetic acid (1%) and then extracted using ethylacetate, evaporate and reconstituted in 50µl acetic acid (1.%) solution. The elution was carried out in reversed phase, using a C18 column in gradient mode. The triple quad operated in Multiple Reaction Monitoring mode, in positive ionization.
The method provided adequate sensitivity for docetaxel and M4, with LODs of 3.0 and 1.0 ng/ml, and LOQs of 5.0 and 3.0 ng/ml respectively. LODs and LOQs of M1-M3 (3.0 and 5.0 ng/ml) did not significantly differ. The method was validated in the range from LOQ to 2000 ng/ml. Several factors that could interfere with extraction efficiency were then evaluated; dryness/reconstitution procedures were optimized in order to minimize the effects. The method was applied to 15 subjects under therapy. Whole blood samples were collected starting from about 5 minutes before docetaxel infusion and up to 6 hours after the end of the administration time. Docetaxel was measured in all the subjects and was detected in all the post-administration samples. Among metabolites only M4 was identified in 10 out of 15 subjects. M4 disappeared three hours after docetaxel administration.
This project represents a novel pharmacokinetic approach for the evaluation of docetaxel therapy. The increasing of method sensitivity, as well as the evaluation of an alternative metabolism pattern of docetaxel could and will be eventually studied, in order to monitor the metabolic path of this drug after administration.

P168. ACUTE DYSTONIC REACTION AFTER COCAINE CONSUMPTION TAINTED WITH HALOPERIDOL: A CLUSTER OF CASES IN BRAZIL

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Dystonia is a disorder characterized by involuntary muscle contractions that cause slow repetitive movements or abnormal postures. The movements may be painful, and some individuals may show tremors or other neurologic features. Acute dystonia has been described after CNS infections (encephalitis), poisoning or adverse reactions to medicines, particularly neuroleptics as haloperidol, chlorpromazine, levomepromazine, atypical antipsychotics and anti-emetics as metoclopramide.
To report a clinical investigation and toxicological screening of a case series of 34 patients that developed acute dystonic reactions, from the same geographic area.
Case Report: During three consecutive days the Campinas Poison Control Center received several phone calls from two distinct hospitals (nearby cities, 42 km apart), reporting a series of patients showing similar signs/symptoms,

compatible with acute dystonia, such as muscle rigidity of upper limbs and torticollis. The patients showed no fever, and all the dystonic features reverted after IV biperiden. All of them denied previous use of any psychoactive or pharmaceutical drugs, and all of them believed that the neighborhood "drinking water" should be responsible for the symptoms. Serum (12 samples) and urine (22 samples) of the patients, and drinking water samples were collected and sent to our laboratory. The urine screening by immunoassay detected cocaine in all patients' samples. A target analysis by LC-MS/MS confirm the cocaine screening of urine and also detected and quantified haloperidol in all urine and serum samples, but not in the suspicious water sample. At the third day, 12 additional patients with the same clinical characteristics, from a nearby city, reported to have been in a rave party and confirmed cocaine use. These 12 patients had also the same acute dystonic reaction which was reverted with benzodiazepines. Unfortunately biological samples of these cases were not collected. All 34 patients were discharged without any sequel.
The sample preparation was based on liquid/liquid extraction with methyl tert-butyl ether at pH 7.0 prior to injection into the LC-MS/MS system. Analyses were performed in MRM (positive mode), with the identification by retention time and two MRM transitions ratio. The haloperidol quantitative analysis showed the following serum levels: highest level: 0.25 ng/mL; lowest: 0.1 ng/mL; mean: 0.17 ng/mL (toxic level > 50ng/mL) and urine levels: highest level: 1200.6 ng/mL; lowest: 1.34 ng/mL; mean: 264.4 ng/mL in samples collected in different hours/days after exposure (LOQ:0.1 ng/mL for serum and urine).
Laboratory results showing the presence of haloperidol in biological samples of the 22 patients, who supposedly had consumed cocaine from the same drug dealer, indicates a new and dangerous way of street tainting cocaine.

P169. INTOXICATION WITH A MIX OF DESIGNER DRUGS – A CASE REPORT

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A 38-year-old male with a known history of epilepsy and depression, suffering from dependence syndrome and personality disorder, was admitted to the intensive care unit following ingestion of an unknown drug in a supposed suicide attempt. On admission, the patient became more and more somnolent and unresponsive to pain stimuli (GCS 4-5), with dilated pupils, sinus tachycardia and marked dehydration.
Urine and stomach content were collected at admission to be screened for drugs of abuse and medicinal drugs. After admission, the patient's next of kin presented five small grip seal plastic bags containing different powders and labeled with three-letter code names. As it was reasonable to believe that the patient had consumed some of the aforementioned powders, the powders were sent along with urine and stomach content to our laboratory for toxicological analysis.
An easy and rapid sample preparation technique was applied for extraction of urine and stomach content. Samples were spiked with an internal standard (nalorphine) and extracted with ToxiTubes® A (Agilent). After centrifugation, organic layers were collected, transferred into vials, evaporated to dryness under mild nitrogen flow and the residue dissolved in 50 µL of methanol and analysed by GC-MS (Shimadzu GCMS-QP2010 Plus). A small amount of powder material was diluted in methanol and injected directly in the GC-MS instrument. Separation was performed on an Agilent DB-5ms column (30 m x 0.25 mm ID x 0.25 µm) using 1.2 mL/min helium as the carrier. The inlet was operated at 280°C, 1 µL injections were made in split mode with a 13:1 ratio. The oven was programmed to start at 70°C, ramping 15°C/min to 200°C; then ramping 7°C/min to 280°C followed by a 10 min hold. The GC-MS instrument was operated in the SCAN mode.
Obtained spectra (EI) were evaluated against SWGDRUG library (Shimadzu format). Five different designer drugs were identified in the powder material, including synthetic cannabinoids (AB-CHMINACA, AB-FUBINACA) and synthetic cathinones (alpha-PHP, alpha-PVP, 4-CMC). Both cannabinoids and cathinones were also detected in the stomach content along with prescription drugs (diazepam, quetiapine), while urin tested positive for cathinones and prescription drugs (diazepam, quetiapine and fluoxetine).
Designer drugs remain a worldwide problem with increasing prevalence. Furthermore, their continuous widespread availability (in here presented case, the patient is believed to have purchased the drugs via the Internet from a clandestine manufacturer in China) and unpredictable effects, especially when used in combinations with prescription drugs or other designer drugs, make them an important public health and safety issue. While the patient described here firmly denied attempting to commit suicide, his intoxication nearly proved to be fatal. Toxicological analysis is an indispensable tool in the identification of these novel drugs, as patients commonly present with non-specific signs and symptoms when intoxicated by them.

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P170. **STUDY OF THE INCIDENCE OF NEW PSYCHOACTIVE SUBSTANCES IN SPAIN IN 2014**

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The last reports of the European Monitoring Centre for Drugs and Adictions (EMCDDA) and the United Nations Office on Drug and Crime (UNODC) highlights the growth of the market of the New Psycoactive Substances (NPS) over the last years, increasing the number, type and the availability of these substances, producing a significant public health problem with non-fatal intoxications and deaths. One of the major problems of these is the speed at which they appear, their open sale, and that there is little or no information on their effects and harms.
The aim of this study is to review the NPS data obtained from the analysis performed in our laboratory during the period of 2014, both in the area of analysis of seized materials and in the area of Forensic Toxicology, and identify and evaluate the most relevant information relating to them.
Statistical analysis was performed from the data of the samples collected by police and forensic pathologists. The analysis of these samples were performed by GC-MS, HPLC-DAD and FTIR.
About 26% of the 30,000 samples of seized material received contain NPS. The more frequent substances were found in the group of Synthetic Cathinones (37%): 3,4-methylenedioxypyrovalerone (MDPV), Methylone, 4-methylmethcathinone (mephedrone), 4-Methylethcathinone (4-MEC), Pentedrone and Ethylone. Secondly, the most relevant group were Phenethylamines (35%): 2C-E, 2C-I, 4-Fluoroamphetamine, 4-Fluoromethamphetamine, 2,5-dimethoxy-4-chloroamphetamine (DOC) and 25-NBOMes. Thirdly (25%) it could be found Ketamine and Methoxetamine. Finally, less than 3% of NPS received are being reclassified in new drug families, based on chemistry, for example: Opioids (Dextro/Levometorfane), Aminoindanes (2- Aminoindane), Piperidines and Pirrolidines (Ethylphenidate), Benzodiazepines (Fenazepam, Diazepam) and Plants and Extracts (Salvia divinorum, Ayahuasca). The absence of Synthetic Cannabinoids should be noted, which is mainly because Spain is one of the main routes for cannabis produced in Morocco (Africa) and the increasing growth of marijuana plants that are genetically modified to have a higher THC content. Also, the detection of two cases in the area of Forensic Toxicology it is noted. One from a living person intoxicated with Methylone and another from a postmortem case with PVP and 4-MEC.
This study provide an overview of the cualitative and cuantitative sample composition of the Spanish illicit market, which shows that it is very dynamic, motivated by the control legislation. In previous years Synthetic Cathinones and Synthetic Cannabinoids were found in different mixtures and distributions, but due to the change of the law, they have been substituted or replaced by other substances with similar effects, such as Methoxetamine. Also, there have been appeared new different families of substances not monitored by EMCDDA, as mentioned above. The evaluation of these results provides an interesting source of updated information on the NPS consumed in the area of influence of our laboratory and its potential application to solving cases of Forensic Toxicology.

P171. **CHANGING THE STRUCTURE OF CLASSICAL ILLICIT AND STIMULANT DESIGNER DRUG CONSUMPTION BETWEEN 2008 AND 2014 AMONG SUSPECTED DRUG USERS IN SOUTH-EAST HUNGARY**

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Csongrád County (South-East Hungary) is neighboring the Serbian and Romanian border through which a considerable part of illicit drugs are smuggled to Hungary from the Balkan. Before 2010 this route determined the illicit drug supply of South-East Hungary but the escalated use of designer drugs has changed the structure of the black market.
The aim of this work is to present the main changes of illicit and stimulant designer drug consumption among suspected drug users between 2008 and 2014 in this area.
Urine and/or blood samples of 2658 suspected drug users were analyzed by GC-MS following liquid/liquid extraction for the most frequent illicit and licit drugs and for stimulant designer drugs (SDDs) between 2008 and 2014.
Since 2011 the frequency of cannabis consumption decreased by 15-20%. Between 2010 and 2012, the prevalence of SDDs showed a marked elevation which accompanied by a decreased frequency of classical amphetamines (AM, MA, MDA, MDMA). In 2013-14 an opposite tendency was observed. The most frequent SDDs were: mephedrone in 2010; 4-MEC, methylone, MDPV, 4-FMC, and 4-FA in 2011; pentedrone, 3-MMC, and 2-MPA in 2013; pentedrone and 3-MMC in 2014. In the biological samples SDDs were more frequent in combination than alone.
The structure of SDD abuse aligned with the legislative changes. Substances that were defined as illicit by the court disappeared from the local black market (e.g. 4-FMC, 4-FA) or their frequency of use dropped. To avoid legislation these substances were replaced with new analogs. SDDs were often combined with amphetamines or other SDDs to modulate entactogenic and stimulant effect, or with benzodiazepines or THC to counteract overstimulation. The decreased frequency of cannabis since 2011 is due to the spreading of synthetic cannabinoids while the temporary decrease in the frequency of classical amphetamines was probably an attempt by the users to replace them with SDD analogs.

P172. **DEMONSTRATION OF SCREENING OF OVER 300 COMPOUNDS IN URINE USING TRIPLE QUADRUPOLE MASS SPECTROMETER AND SOFTWARE FOR RAPID DATA ANALYSIS**

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Forensic toxicologists need tools for rapid and confident screening for large numbers of compounds. Triple quadrupole mass spectrometers are becoming the instruments of choice in many forensic toxicological laboratories, supplanting traditional immunoassays because they offer better specificity within compound classes, can test for a wider range of compounds, and can easily add more compounds to the test menu. In order to generate results as fast as immunoassays, mass spectrometry methods must contain a larger number of analytes in one analytical run. Additionally, software is needed for easy and rapid data analysis of the large panels.
Demonstrate a screening method for over 300 compounds on a triple quadrupole mass spectrometer with associated data analysis software.
Samples were prepared either by urine dilution or enzymatic hydrolysis followed by further dilution; both schemes had a final dilution of 30-fold. Tolbutamide-d9 was used as a single internal standard. Samples were introduced to a Thermo Scientific™ TSQ Endura™ triple quadrupole mass spectrometer after gradient chromatographic separation. One to three SRM transitions in both positive and negative ionization mode were collected for each of the over 300 analytes. The fast scanning speed and polarity switching of the TSQ Endura allows more transitions, and thus more compounds, to be packed into a single analytical run. Data was acquired with Thermo Scientific TraceFinder™ software, version 3.2 and analyzed with Thermo Scientific ToxFinder™ software, version 1.0. Screening performance was assessed by analyzing compounds in pools of ten each at a single concentration of 100 ng/mL. Additionally, donor samples of known composition were also analyzed. ToxFinder software identified compounds based on retention time, number of product ions detected and product ion ratios. Semi-quantitation can be performed either by using a single point calibrator or by using internal standard ratio.
Over 90% of the compounds in the spiked pooled samples tested screened positive for at least one SRM transition. Many of the negatively screened compounds have higher cutoff limits, and so it was not unexpected that they were not reported as positive. As expected, more compounds were reported as positive with one confirming SRM transition, with fewer compounds being reported as positive as the number of confirming SRM transitions increased. More confirming transitions offer better specificity, but come at the price of reduced sensitivity.
We successfully screened for a large panel of compounds. This combination of instrumentation and software are a fast and reliable platform for screening compounds in a forensic toxicological setting.

P173. **TRENDS OF ETHANOL AND ILLICIT DRUGS USE AMONG DRIVERS INVOLVED IN TRAFFIC CRASHES IN CAMPANIA (ITALY) DURING 2009-2014**

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In Italy studies regarding the toxicological findings among drivers hospitalized after a crash, are still insufficient and not homogeneous both in the sampling and in the analytical approach.
This study assesses trends in the intake of ethanol and/or illicit drugs among drivers (n. 1058) involved in road accidents and admitted to the emergency unit of 16 hospitals of the Campania Region (Italy), from 2009 to 2014.
To demonstrate the violation of the Road Safety Law, toxicological analyses were requested by police at the Forensic Toxicology Unit of the Second University of Naples for 434 blood samples. The urine (n. 624) was collected only when the sampling of blood was denied by drivers. A GC/HS-FID methodology was applied to testing the blood alcohol concentration (BAC) in 341 cases of suspect driving under the influence of alcohol (DUI) and in 93 cases of recent use of alcohol associated with illicit drugs (DUID). Validated methods (GC/MS or LC-MS/MS) for amphetamine and analogous,cocaine, opiate, benzodiazepines (n.35 BDZ), methadone, barbiturate, buprenorphine and cannabis metabolites were applied to blood or urine specimens to confirm the presence of psychoactive drugs. Trends in the use of alcohol and drugs among drivers in the years 2009-2011 and 2012-2014 were compared. The BAC greater than 0.5 g/L (legal limit in Italy) was confirmed in 92% of drivers with a significant increase in the period 2012-2014 (n. 231) compared to 2009-2011 (n. 100). The higher incidence of crashes involving drivers with BAC> 1.5 g/L, grows up from 40% to 58% from 2012 to 2014. In DUID cases (n.93) accidents occurred also with a lower BAC involving almost 42% of drivers with BAC in the range 0.8-1,5 g/L and 36% with BAC >1,5 g/L. In blood of DUID cases THC (35%), poly-drugs (31%) and Cocaine (26%) were the most confirmed substances whereas Opiate, BDZ and Methadone were present in < 5 % of cases. In urine THC-COOH was the most frequently identified compound alone (mean 31%) or in combination with other illicit drugs. In fact in drivers, positive for more than one drug (42% during 2009-2011 decreased to 22% in 2012-2014), THC-COOH was almost always present. Interestingly the incidence of Cocaine and BDZ, as only drug used, was duplicated in the years 2012-2014 compared with 2009-2011. Moreover in the accidents due to poly-drug use the cocaine was the drug prevalently confirmed together to THC-COOH.
The study shows, since 2012 in Campania (Italy), an increasing involvement of Cocaine and Cannabis, alone or in combination with alcohol and other drug, in causing road traffic accidents. Therefore, in order to collect significant data for the future preventive policies, the use of pharmaceuticals, illicit drugs and alcohol should be routinely checked in all crashes using a forensic analytical approach.

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P174. DETECTION OF DRUGS IN 275 ALCOHOL-POSITIVE BLOOD SAMPLES OF KOREAN DRIVER

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Since driving under the influence of drugs(DUID) is as dangerous as drink-driving, many countries regulate DUID by law. However, the law against use of drugs while driving is not established yet in Korea. Furthermore, there are little reported cases related to DUID.
In order to investigate the type and frequency of drugs used by drivers in Korea, we analyze controlled and non-controlled drugs in alcohol-positive blood samples.
Total 275 blood samples were taken from Korean drivers, which were positive in roadside alcohol testing. The analytical strategy was composed of following three steps: First, alcohol in blood samples were confirmed and quantified by gas chromatography. Second, for a preliminary test, blood samples were screened for controlled drugs by Evidence investigator™(Randox, U.K.) which is a biochip array analyzer based on immunoassay. Final confirmation for positive samples on immunoassay was performed by GC-MS. Third, drugs related to DUID were examined in 275 blood samples. Total 49 drugs including controlled drugs, antidepressants, 1st generation antihistamines, dextromethorphan, nalbuphine, ketamine, etc, were selected for DUID test. Blood samples were prepared by solid-phase extraction using RapidTrace™(Zymark, U.S.A). After trimethylsilyl(TMS) derivatization, samples were analyzed by GC-MS. For a rapid detection of 49 drugs, we developed automated identification system called "DrugMan", a newly developed macro modules with modified Chemstation data analysis. In addition to the 49 drugs, general drugs were also examined from the TMS derivatized blood samples using GC-MS library provided by the Agilent .
Concentrations of alcohol in 275 blood samples were ranged from 0.011 to 0.249 % (average 0.119 %). Six specimens showed positive results by immunoassay: one methamphetamine and five benzodiazepines I. By GC-MS confirmation, only benzodiazepines (diazepam, nordiazepam and temazepam) in 4 cases were identified, while methamphetamine and benzodiazepine in 2 cases were not detected from the presumptive positive blood samples. Therefore, they were determined as false positive. Using DrugMan, three drugs were detected; chlorpheniramine(5)*, dextromethorphan(1) and doxylamine(1). In addition, ibuprofen(1), lidocaine(1) and topiramate(1) were also detected as general drugs in 275 blood samples by the Agilent GC-MS library. (* means the frequency)
This study examined the type and frequency of drugs used by Korean drivers. For drug screen, 49 drugs related to DUID were selected and DrugMan, a newly developed automated identification system, enabled it to detect these drugs within one minute. The frequency of drug abuse by Korean drivers was relatively low and total 14 cases were positive in 275 blood samples with a ratio of 5 %. The highest frequency of drug was chlorpheniramine followed by benzodiazepines. However, these results were limited to alcohol positive blood samples, thus, it is necessary to analyze more samples including alcohol negative blood to get the detailed information.

P175. HAS PREVIOUS ABUSE OF FLUNITRAZEPAM BEEN REPLACED BY CLONAZEPAM?

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For many years, flunitrazepam was the benzodiazepine of choice among problematic drug users in Norway. The large extent of misuse resulted in reclassification of flunitrazepam to the highest scheduling level (class A), the same classification as for strong opioids and other narcotics. At the same time, the diversion of Rohypnol® from licit manufacturers in Eastern Europe to the illegal market was stopped. Now, flunitrazepam is detected in only a small number of forensic toxicology cases each year. However, the number of clonazepam (class B) findings has increased.
To investigate the increase in clonazepam findings in blood samples from suspected drug- impaired drivers and assess whether this was related to increased prescription or drug trafficking.
We used data from three sources to study the changes in the use of clonazepam: 1: Presence of clonazepam and flunitrazepam in blood samples collected from Norwegian suspected drug-impaired drivers, 2: Sales numbers (legal market) for clonazepam, extracted from the Norwegian Prescription database (NorPD); and 3: Specific seizures (illegal market) for clonazepam in Norway.
In 2004, 13.0% of the analysed blood samples from suspected drug-impaired drivers contained clonazepam, whereas this proportion had increased to 27.7% in 2013. In the same period, the frequency of flunitrazepam in drugged drivers decreased from 16.6% in 2004 to 3.2% in 2013. The number of clonazepam prescriptions decreased, while the number of seized tablets containing clonazepam increased considerably from 2004 to 2013.
For the last 10 years, a significant increase in the illegal use of clonazepam has been seen among drug-impaired drivers, now replacing flunitrazepam as the most used illegal benzodiazepine in Norway.

P176. **ROADSIDE SURVEY ON ALCOHOL AND DRUG USE AMONG DRIVERS IN THE ARCTIC COUNTY OF FINNMARK (NORWAY)**

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The relative number of arrests for driving under the influence of alcohol or drugs in Finnmark County is higher than in other parts of Norway. There is also rapidly increasing road traffic across the border from Russia; thus, a number of drivers with a different drinking culture are entering into Norway. For those reasons, the prevalence of alcohol and drugs among drivers might be different from the rest of Norway.
To investigate the prevalence of alcohol and drug use among drivers in normal road traffic in Finnmark County by analysing samples of oral fluid.
In collaboration with local police, drivers were selected using a multistage cluster sampling procedure (selection of roads, time intervals, and drivers within each interval). Age, gender, time and geographical site were recorded. Samples of oral fluid were collected using the Quantisal device. The samples were analysed for alcohol with an enzymatic method and for 12 illicit drugs and 16 medicinal drugs and some metabolites using UPLC-MS/MS. Sample collection started September 2014 and is planned to end one year later; the aim is to collect samples from 3000 drivers.
About 1400 samples were collected during the first 6 months. The participation rate was 95%. Preliminary findings showed that 6.6% of the samples were positive for alcohol or drugs. Alcohol was found in 0.3%. The most prevalent drugs were zopiclone 1.5%, THC 1.4%, cocaine/benzoylecgonine 0.8%, codeine 0.8% and diazepam 0.7%. 0.4% was positive for phenobarbital or phenazepam, which are more commonly used in Russia than in Norway.
The results so far suggest that the prevalence of alcohol and drugs in samples of oral fluid from drivers in the northernmost part of Norway is similar to the findings in previous Norwegian roadside surveys, which covered geographical regions further south. However, the prevalence of cocaine and Russian medication might be higher. The final conclusion will be drawn when the study has been completed.

P177. **VISUAL EFFICIENCY IN DRIVERS CONVICTED OF DRIVING UNDER THE INFLUENCE OF ALCOHOL AND DRUGS**

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Alcohol and drugs users show abnormalities on measures of brain functioning - linked to change in neurocognition over time - that probably contribute to traffic crash risk and recidivism in risky behaviour.
The aim of the study was to evaluate the presence of cognitive and psychomotor impairment in subjects convicted of driving under the influence (DUI) of alcohol and drugs.
37 DUI offenders, submitted to toxicological and medical assessment for driving license re-granting, were analyzed – in sober condition - through a psychometric evaluation with an automatic eye tracking test (Tobii T60) in order to study their visual efficiency. Visual pattern of traffic images (driving in heavy rain and poor light, road with poor visibility due to fog and a traffic incident) was evaluated. The Choice Reaction Time (CRT), a short -term memory task, was also carried out. The results were related to subjects' data (demographic data, recidivism for DUI, main markers of alcohol abuse) and statistically analyzed (Pearson correlation).
All subjects (32 men and 5 female; mean age 35 y.o. – range 25-53) were convicted of DUI of a combination of alcohol and drugs; alcohol/cocaine and alcohol/cannabis were the most common co-finding. In visual efficiency evaluation, correlations were found between: a) intake of legal drugs (causing impairment) the day before, younger age, low level of education and longer fixations just in the center of image ($r=0.043$ $p=0.043$; $r=-0.419$ $p=0.021$; $r=-0.461$ $p=0.010$, respectively); b) increase of serum alkaline phosphatase and reduction of the number of areas of interest (AOI) explored ($r=-0.328$ $p=0.077$); c) serum alkaline phosphatase level and number of fixation points ($r=-0.532$ $p=0.001$); d) female gender and shorter fixations in the center of the image ($r=-0.473$ $p=0.008$); e) female gender and the number of AOI explored ($r=-0.423$ $p=0.020$). The recidivists explored few AOI ($r=-0.320$ $p=0.084$) and watched more time the center of the image ($r=0.367$ $p=0.046$). In CRT performance, a weak correlation between number of errors and intake of legal drugs (causing impairment) the day before test was found ($r=0.313$ $p=0.092$), while a statistically significant linear correlation between increase of serum alkaline phosphatase and reaction times emerged ($r=0.391$ $p=0.032$).
This is the first study that evaluate the psychomotor and cognitive performance, through the visual pattern analysis and short-term memory evaluation, in subjects convicted of DUI of alcohol and drugs in sober conditions. Data suggest that residual effect of cognitive impairment can be revealed in this heterogeneous population. Emerging correlations seem also in according to previous study on increase of serum alkaline phosphatase highlighted in chronic usage and dependence of cannabis and other substances. Psychometric assessment of poly-substance abusers may contribute to identify the drivers with potential brain injury. Research is needed to further define whether this cognitive impairment can be related to traffic crash risk.

Poster abstracts

P178. OPIATE ABUSE IN DUI URINE: SCREENING OF 6-MONOACETYLMORPHINE IN URINE OF DRIVERS BY A NEW IMMUNOASSAY METHOD

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Painkiller opiates are often administered to the victims of road accidents: among these drugs, a frequently used one is morphine, which is sometimes administered before the taking of biologic samples requested by the traffic control authority. In such cases, a test capable to discriminate the opiates metabolites in the analyzed samples may be useful to distinguish heroin users from patients treated with morphine.
The aim of the present study was the validation of an immunoassay method for the semi-quantitative determination in urine of 6-Acetylmorphine (6-AM), an heroin metabolite which presence confirms the illicit use of heroin rather than the assumption of therapeutic morphine. By subministration of morphine, in fact, no positivities for 6-AM would result in biological samples.
In this study we adopted the Emit® II Plus 6-AM Assay on Siemens H.D. ADVIA® 1200 analyzer. Semi-quantitative calibration curves, from 5-10-15-20 ng/mL ready-to-use calibrators, were prepared by adapting volumes of kit reagents and of sample. The validation protocol adopted required the evaluation of linear calibration, precision, accuracy, carry-over, stability-on-board. A pool of 68 samples, of which 56 resulted positive and 12 negative for opiates, were collected from subjects who were involved in road accidents and from deceased subjects who underwent judiciary autopsy; all the samples were previously tested by Emit® II Plus Opiate Assay on the same ADVIA® 1200 analyzer and confirmed by routinely gas chromatograpy/spectrophotometry (GC/MS) method for the presence of morphine, codeine and 6-AM.. All samples were re-examined by Emit® II Plus 6-AM Assay and the results were evaluated by a Golden Test of Very Positive (VP), False Positive (FP), Very Negative (VN) and False Negative (FN).
No False Positive nor False Negative were registered in the study; sensibility and specificity of the assay resulted 100%; Predictive Positive Value and Predictive Negative Value were always 100%. Precision on level 2 (10 ng/mL) obtained a SD = 0.7 and a CV% of 8.1%, and at level 3 (15 ng/mL) obtained a SD = 0.5 and a CV% of 3.8%: both CV% were largely included in the range of acceptability (<10%). Accuracy on level 2, level 3 and level 4 (20 ng/mL) was collectively within <20% of error; stability-on-board was established at 14 days. In the real samples examined, the Emit® II Plus 6-AM Assay presented positive results for 6-AM (> 10 ng/mL) only in subjects who had taken heroin, as confirmed by GC/MS, and negative results for 6-AM (<10 ng/mL) in subjects resulted at confirming test by GC/MS positive for morphine, or morphine and codeine, but not positive for 6-AM.
An immunoassay method for differentiation of opiate metabolites in urine has been validated and, when applied to real samples, it is useful to differentiate heroin from morphine intake in cases of DUI urine analysis.

P179. EFFECTS OF FLUORIDE CONCENTRATION ON THE STABILITY OF COCAINE IN BLOOD COLLECTION TUBES

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The hydrolysis of cocaine in blood samples after collection is a commonly known problem in forensic toxicology. As already presented by R. Baselt, and confirmed by numerous other publications, the addition of fluoride to the blood tube significantly reduces this degradation. Therefore, in cases where the detection and blood concentration of cocaine in the blood sample is crucial for the interpretation, numerous recommendations on the type of blood collection tube have been given. Rees et.al have studied the effects of two different fluoride concentrations in the blood collection tube using horse blood, however so systematic determination of a fluoride concentration effect nor a systematic determinations of commercially available tubes containing fluoride (and other preservatives) on the stability of cocaine in human blood has been published.
The aim of the study was to systematically evaluate commercially available tubes containing fluoride as well as increasing fluoride concentrations on their effects of cocaine stability in blood samples.
Blank blood was fortified with a cocaine concentration of 1000 ng/L and stored at room temperature in 4 different commercial blood collection tubes containing fluoride over a time period of 5 days. Additional blood (prepared identically) was also stored in blood collection tubes containing increasing amounts of sodium fluoride from 1-50 mg/mL. All samples were analysed using LC-MS (ABSciex 3200 Q-Trap) after protein precipitation monitoring the concentration of cocaine, benzoylecgonine, ecgonine methyl ester and ecgonine.
Cocaine degraded in fluoride containing tube at a rate similar to published research. In contrast to the published data using horse blood, no significant changes were observed for different fluoride concentrations. Commercially available tubes containing fluoride showed no significant differences to fluoride tubes prepared at increasing concentrations.
No significant concentration dependence on the degradation of cocaine in blood samples was observed. As previously recommended, blood collection tubes need to contain fluoride in order to have some effects on the cocaine concentrations, however based on this data there is no merit for specific blood collections tubes.

P180. INTERFERENCE OF ANESTHETICS IN BLOOD ALCOHOL ANALYSIS BY HS-GC/FID: A CASE REPORT

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The presence of ethanol in blood can, when combined with activities such as driving, have serious effects on the life of an individual because of the increased risk of accidents or due to legal consequences. According to good laboratory practice, the chromatographic separation by HS-GC/FID is performed using two capillary columns of different polarity, thus providing a significant change in retention and elution order of ethanol and internal standard, as well as of other volatiles that may arise in routine analysis. The authors present a case of blood alcohol determination on a male person of 28 years old, which intervened in a road accident as a driver. To the individual in question was provided hospital care before the blood collection. The analysis revealed the presence of ethanol, but in different concentrations for each of the columns.
This study highlights the importance of using two chromatographic columns with different polarities in the determination/quantification of ethanol, particularly in the presence of other volatile substances.
The analysis was made using an Agilent 6890N gas chromatograph equipped with a flame ionization detector and coupled to an Agilent G1888 headspace injector of fixed volume (1 ml) (HS-GC/FID). Chromatographic separation was performed using two capillary columns with different polarities (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). Prior to gas-chromatography analysis, the samples, including the calibrators, were diluted 1:10. With this purpose, 100 µL of blood were diluted with 1 mL aqueous solution of n-propanol (100 mg/L), used as internal standard. Lower limits of detection and quantification of ethanol were, respectively, 0.02 g/L and 0.08 g/L, and the method was linear in the concentration range of 0.1- 5 g/L (r2>0.99).
Quantification of blood ethanol in this case revealed a value of 1.56 g/L and 0.34 g/L for each set of column/detector, respectively. According to the law in our country, the result obtained from the first column presented a value considered crime (above 1.2 g/L), while the result from the second column would not originate any penalty (lower than 0.5 g/L). Since there was a good chromatographic peak separation in the second column, we can conclude that these substances co-eluted in the first column. Further clinical data revealed that it was administered at the hospital a volatile anesthetic (sevoflurane), which was later confirmed as the interfering substance in this analysis. Since the use of volatile anesthetics is very common after road accidents, this case demonstrates the importance of using two GC columns with different polarities for an unequivocally identification of ethanol.

P181. ILLICIT DRUGS AMONG DRIVERS, A FIRST TOXICOLOGICAL INVESTIGATION IN ALBANIA

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Road safety is one of the main goals of the World Health Organization. There has been some investigation regarding the influence of alcohol in traffic crashes in Albania (population 3 190 089), but never a research regarding the use of illicit drugs in drivers in our country. This was an initiative of forensic toxicologists to investigate the use of drugs among drivers involved in accidents. By law, the prosecutors are the authority who asks for the toxicological examination specifying the type of analysis they want to be performed.
Albania introduced a "zero tolerance" law on driving under the influence of illicit drugs and alcohol; but in everyday routine only alcohol analyses are required from the police officers responsible for the blood samples taken from drivers involved in road accidents. This study aims to investigate the presence of illicit drugs in this group of road users who already had an accident.
Blood samples from 82 drivers involved in road accidents, during a six month period were obtained from police officers or medico-legal doctors and have been analyzed for alcohol (ethanol) by Head-Space Gas Chromatography. All these samples were also screened for the presence of drugs (medicines and illicit drugs) by immunological methods. The cut-off levels of the first screening were : opiates 250 ng/ml; cocaine metabolite 200ng/ml; cannabinoids 20 ng/ml; amphetamines 300 ng/ml; benzodiazepines 250 ng/ml; tricycle antidepressants 200 ng/ml; barbiturates 200 ng/ml. Positive results after screening were confirmed by means of gas-chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS).
Overall, 82 car drivers were included in the study. 99% were men and 60% of the totals were fatal cases. In 58.5% of the cases BAC was <0.5g/L; in 9.8% of the cases BAC value was 0.5-1 g/L and in 31.7% of the cases BAC exceeded 1 g/l. In total, 13 (15.8%) were positive for illicit drugs and medicinal drugs above the LOQ, The average age was 37 (SD=15.65), but there was not observed any significant statistically difference regarding the average age of non-drug users vs. drug users. Cocaine was found in 1 (1.2%) of drivers, methamphetamine in 1 (1.2%) : MDMA in 2 (2.4%) and benzodiazepines in 8 cases (9.8%) of total drivers who had an accident. 38.5% of drug users group were positive for ethanol too.
The results of this survey revealed that beside alcohol illicit drugs and medicinal ones are present in Albanian drivers. The most frequent identified drug was benzodiazepines group followed by ecstasy.

Poster abstracts

P182. TESTING ALCOHOL RELATED DRIVING IMPAIRMENT: EVALUATION OF AN EASY-TO-USE SMARTPHONE MOBILE APPLICATION.

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The safety of driving vehicles is controlled by applying penalties on incorrect driving behaviors or, with a preventive approach, by hindering from driving the subjects found unfit-to-drive. The latter approach is today almost limited to the use of so called "interlock systems" which are based on a on-the-spot BAC determination. Although widely tested, these systems have so far found limited application. In addition to costs and complexity, an important factor hampering the use of these devices is that their action is limited to alcohol intoxication. The diffusion of smartphones hosting sophisticated mobile applications (Apps) may offer an alternative tool to verify the ability to drive by using simple tests to assess the psychomotor functions.
The aim of the present work was to evaluate the performance of a free accessible smartphone app, named "SAFE Drive", testing psychomotor functions related to ability to drive. The study was performed in the frame of "Safe Drive Project". Fifteen healthy volunteers (7 males, 8 females, 30-35 years old) were enrolled in the testing program.
The "SAFE Drive" app included four sub-tests checking neuromotor reflexes to different visual/auditory stimuli (simple visual, complex visual, simple auditory, complex auditory). The enrolled subjects drunk wine during a meal until a BAC of 0.8 g/L was reached. The BAC was monitored with a breath analyzer. The Safe Drive test was performed by each subject six times before alcohol intake and six times at BAC of 0.8 g/L. The response times before and after alcohol intake were compared by using the Students T test.
The results were evaluated by comparing the mean response time of the group before and after alcohol intake. Moreover, a longitudinal evaluation of response time of each subject was performed. The former analysis showed a statistically significant difference for the simple auditory and the complex visual sub-tests ($p<0.05$). Also the simple visual was not far from the statistical significance. However a much higher statistical significance was obtained in the longitudinal test with a p often <0.01.
The results of the study suggest that the "SAFE Drive" app could be suitable to handily assess the driving impairment related to alcohol intake. Moreover, the much higher significance in the longitudinal test supports the use of this app as an auto-testing device.

P183. COMPARISON OF THE RANDOX® EVIDENCE DRUGS OF ABUSE CUSTOM ARRAY VIII BIOCHIP WITH ACCURATE MASS SCREENING I: OPIATES, GENERIC OPIOIDS AND OXYCODONE 1

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Blood specimens collected in suspected DUID cases in the State of Michigan are routinely screened for drugs by the Michigan State Police using a Randox® Evidence Analyzer and a Drugs of Abuse Custom Array VIII Biochip employing chemiluminescent immunoassay technology imbedded with 14 different antibodies to desired target analytes. Specimens that screened positive for one or more analytes on the biochip were sent to NMS Labs for analysis by liquid chromatography accurate mass screening and confirmation of presumptive positive findings.
The objective of this study was to compare the results obtained between the custom biochip opiate assays (opioid (OPI), generic opiates (OPDS) and oxycodone (OXYC1)) with LC-TOF accurate mass screening and LC-MS/MS confirmation.
Randox® Biochip screening was targeted at 25 ng/mL (morphine) for the OPI assay and 15 ng/mL and 5 ng/mL (oxycodone) for the OPDS and OXYC1 assays, respectively. The cross-reactivities (%) as provided by the manufacturer, varied by assay. The OPI assay primarily targets morphine (100%), codeine (106%), hydromorphone (27%) and 6-acetylmorphine (1214%). The OPDS assay targets oxycodone (100%), hydrocodone (1774%), dihydrocodeine (326%), codeine (763%) and hydromorphone (180%). The OXYC1 assay targets oxycodone (100%) and hydrocodone (34%). The LC-TOF decision points were codeine (15 ng/mL), morphine, hydrocodone, dihydrocodeine, oxycodone, oxymorphone (10 ng/mL each) and 6-acetylmorphine (2.5 ng/mL). For LC-MS/MS confirmation the reporting limits were one-half to one-tenth of the LC-TOF decision points.
A total of 1858 blood specimens were tested. The number of cases positive by both the Biochip and LC-TOF were 461, 962 and 603 for the OPI, OPDS and OXYC1 assays, respectively. There were 118 positive opiate Biochip assays (from 95 unique cases) that were not positive by LC-TOF. However, with the exception of 6 of these cases (false positives), all demonstrated evidence of opiate(s) below the LC-TOF decision point. 12 cases positive by LC-TOF that did not screen above the threshold of the biochips (false negatives) had elevated immunoresponses except for three cases where only oxymorphone was present. The percent agreement between the OPI, OPDS and OXYC1 assays and the LC-TOF screen was 97.4%, 95.6% and 99.5%, respectively.
All positive results by LC-TOF were confirmed by LC-MS/MS. When comparing the quantitative results for the analytes with their expected cross-reactivity, it was found that both the OPI and OXYC1 chips had greater cross-reactivity to hydrocodone and the OPDS chip had greater cross-reactivity to hydromorphone and morphine than were expected.
A total of 1858 blood specimens analyzed on a Randox® Evidence Analyzer with a Drugs of Abuse Custom Array VIII Biochip were evaluated for the opiate immunoassays. The combined sensitivity and specificity across the three opiate assays were 88.9% and 98.8%, respectively. If the 89/95 Biochip positive cases with an LC-TOF response for an opiate below the decision point had been analyzed directly by LC-MS/MS, the sensitivity of the combined chips could be as high as 99.2%.

P184. **DRINK-DRIVING TREND AND FATAL CASES IN ALBANIA**

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A lot of people lost their lives in road accidents each year all around the world. Many investigations have been done to estimate the percentage of fatalities on European roads influenced by alcohol; but there are so few data regarding our country, so this paper presents a toxicological investigation to assess the presence of alcohol among Albanian drivers. Road accidents had been increased recently in Albania and the number of fatal cases, also. This paper investigates recent trends in drink-driving and related fatal cases during last five years (2010-2014) in Albania. In 2012, Albania introduced a "zero tolerance" law on driving under the influence of alcohol; but is it followed by a decreased number of road crashes and drink-drivers?! This is a five years study, January 2010-December 2014, of the Laboratory of Toxicology, in the Institute of Forensic Medicine in Albania, responsible for all the country, where there are analyzed all blood samples of people involved in road accidents, fatal and non-fatal ones. A total of 830 blood samples taken from people just after the accident or victims during the autopsy, were analyzed for alcohol by gas chromatography. Alcohol analysis was performed in whole blood samples utilizing a GC headspace method, using a Shimadzu GC Head Space QP 2010.
From 830 persons involved in road accidents, 385(46.4 %) were fatal cases. Blood alcohol concentration was detected in values of above 1 g/l in 281 (33.9%) cases from which 49.8% were fatal cases, 0.5 ≤BAC< 1 g/L in 85 cases (10.2%) where 43.5% of them were fatal; 0>BAC≤0.5 g/L in 80 (9.6 %) cases where 50% were fatal; , BAC=0 g/L in 384 (46.3%) cases. Subjects with positive BAC were more frequently men 98.8% [p=0.003] statistically significant.70% of the positive BAC group were car drivers, and 40% of these road crashes have happened during time interval of 18:00 till 24:00 [p=0.026] statistically significant. Data were analyzed by means of binary logistic regression. Since 2010 the percentage of drink driving during five years declined slightly from 15.2% to 13% in 2011 and then increased to 17.3% in 2012; to 20% in 2013 and reached 34.5% in 2014.
The magnitude of alcohol related accidents has been increased in last five years in Albania, even the new regulation on drink-driving have not improved the situation.

P185. **TRENDS IN DRIVING UNDER THE INFLUENCE OF DRUGS AND BLOOD DRUG CONCENTRATIONS OF COMMON DRUGS OF ABUSE IN IMPAIRED DRIVERS IN THE STATE OF PENNSYLVANIA, USA**

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In forensic toxicology, reported drug concentrations derived from a large dataset are a key to make an assessment in cases. To review blood drug concentrations and determine the frequency and the drug trends analyzed for DUID investigation in Pennsylvania, USA for five years.
From January 2010 through December 2014, NMS Labs analyzed 34,617 DUID cases for drug toxicology from PA law enforcement agencies. Blood samples were screened by ELISA and confirmed by GC-MS or LC-MS/MS. Of the submitted cases, 23,127 cases were also analyzed for the blood alcohol concentration.
The compiled data demonstrated 71.4% (n=24,701) were screened positive for at least one drug class and 68.2% (n=15,772) were confirmed positive for ethanol. Additionally, positivity rates for the confirmatory analyses were examined: phencyclidine, benzodiazepines, and amphetamines (>95%), barbiturates (92.5%), methadone (84.6%), cannabinoids (79.4%) and cocaine/metabolites (78.2%).
Among 24,701 cases screened positive for drugs, cannabinoids (69.0%, n=17,046) was the most prevalent, followed by CNS-depressants (opiates; 30.6% and benzodiazepines; 23.7%) and CNS-stimulants (cocaine/metabolites; 13.6% and amphetamines; 8.2%). The consistent frequency of cannabinoids confirms cannabinoids as the most popular drugs among DUID population.
Frequency among each drug class and blood drug concentrations were also studied. Seventeen commonly encountered drugs and metabolites were evaluated. In those with multiple analytes (e.g., opiates, benzodiazepines), those with the highest frequency among the drug class were benzoylecgonine (99.5%), Delta-9 Carboxy THC (97.9%), amphetamine (85.9%), Delta-9 THC (82.0%) and butalbital (80.0%). Among the benzodiazepine panel, alprazolam (62.8%) was the most prevalent, followed by clonazepam (29.6%) and nordiazepam (25.8%). For opiates, morphine (48.1%) was the most prevalent, followed by oxycodone (41.2%), hydrocodone (15.6%) and codeine (8.4%). 6-Monoacetylmorphine was found at 5.4%.
Blood drug concentrations (mean, median, and range [all in ng/mL]) for several compounds were examine: Delta-9 THC (6.2, 4.1, 1.0-140); 6-monoacetylmorphine (16.1, 4.1, 1.0-780); oxycodone (95.3, 54.0, 5.1-1300); alprazolam (79.4, 58.0, 5.0-1600); cocaine (84.6, 56.0, 20.0-1600); methamphetamine (236, 140, 6.20-4500); methadone (253, 210, 50-1300); and Phencyclidine (45.6, 43.0, 5.3-170).
The mean and median concentrations for five representative compounds (Delta-9 THC, Alprazolam, Clonazepam, Cocaine and Methamphetamine) increased from 2010 to 2014. For instance, in 2010 the mean and median concentrations for alprazolam were 65.7 ng/mL and 47.0 ng/mL, respectively. By 2014, these concentrations were 76.5 ng/mL and 53.5 ng/mL, respectively.
Finally, the drug trends among the opiates class was investigated. 6-Monoacetylmorphine and morphine frequency increased over the 2010-2014 period, while oxycodone and hydrocodone decreased in that time frame.
This review demonstrates that during the studied period 1) drug positivity rate remained consistent, 2) the same drugs were frequently encountered, 3) drug concentrations increased and 4) for opiates, there was a shift in popularity from oxycodone and hydrocodone to morphine due to heroin use.

Poster abstracts

P186. NEW BRAZILIAN MANDATORY HAIR DRUG TESTING LAW FOR PROFESSIONAL DRIVERS IN THE WORKPLACE: IGNORING STANDARDS ADOPTED BY OTHER COUNTRIES

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There has been a global increase in the use of workplace drug testing as a deterrent to psychoactive substance use by workers. In Brazil, since 1992, many companies have adopted drug testing programs for the workplace, following American model of a federal drug free workplace, using urine as the main sample to detect drug use. Nevertheless, in 2015, the Brazilian government enacted a controversial law stating mandatory "wide detection window" (minimum of 90 days) test for drugs based on scalp, other hair and/or nails prior to receiving a commercial drivers license and at the time of resignation/retirement of a professional drivers. Urine and other biological samples are not mentioned in this new legislation.
The objective of this project is to discuss the new Brazilian law compared with the practices from other countries on the subject.
A review of the scientific literature, legislations and guidelines from other countries, including recommendations from scientific associations about workplace drug testing was performed.
The United States has the longest experience with workplace drug testing as a mode of deterrence. The Substance Abuse and Mental Health Services Administration (SAMHSA) requires the use of urine as sample to be used in the Mandatory Guidelines for Federal Workplace Drug Testing Programs. The addition of alternative specimens (hair, oral fluid, and sweat patch specimens) that could complement the existing urine drug-testing program is still under evaluation and discussion. The European Workplace Drug Testing Society (EWDTS) guidelines allow the collection of three samples: urine, oral fluid and hair. In fact, the relatively recent introduction of workplace drug testing legislation in some European countries considers the use of urine as the primary sample for testing to monitor drug use in the workforce.
The new mandatory Brazilian drug testing law based exclusively on the analysis of hair and/or nails does not find parallel application in any other legislation in the world. As the drug screening is expected to be applied only once in a two and a half year interval, abstinence periods could circumvent the desired control effectiveness of the law. The application of this new law in the future will negatively affect the existing workplace drug testing programs in Brazil.

P187. METHIOPROPAMINE IN BLOOD SAMPLES FROM DRIVERS SUSPECTED OF BEING UNDER THE INFLUENCE OF DRUGS

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Methiopropamine (MPA; 1-(thiophen-2-yl)-2-methylaminopropane) belongs to the new psychoactive substances (NPS) which have emerged on the drug marked over the last years. MPA appeared in 2011 and is an analogue of methamphetamine, sold as e.g. "Slush Eric" and "Blow". It is reported to have similar effects as methamphetamine, but the toxicity in humans is not known. Three fatal cases involving MPA have been reported, and one analytical confirmed intoxication case has been published. The prevalence of recreational use of MPA is unknown, and no studies have reported the prevalence in driving under the influence of drug (DUID) cases.
The aims of the study were to investigate the frequency of MPA in DUID cases received at our institute during a 12 week period, and compare the analytical findings to the results from a clinical test of impairment performed by a physician shortly after the driving and, if possible, identify the impairing effects of MPA.
All DUID from Norway are analysed at Division of Forensic Sciences at the Norwegian Institute of Public Health (NIPH). Analysis of MPA has been included in this routine screening since July 10th 2014. Whole blood samples were collected for a period of 12 weeks late 2014 and analysed in on a UPLC-MS/MS, applying an Acquity UPLC® BEH phenyl-column using gradient elution with a mobile phase consisting of 5 mM ammonium bicarbonate buffer pH 8 and methanol. Sample preparation was performed using liquid-liquid extraction with ethyl acetate/heptane (4:1) after adding the internal standard amphetamine-d11 and ammonium borate buffer pH 11. The extracts were evaporated to dryness and reconstituted in 100 µL 5 mM ammonium bicarbonate/methanol mixture before analysis. The samples were analysed for 42 different psychoactive substances, and MPA was detected in ten DUID cases (0.8% of the cases), only from male drivers. Other drugs were detected concomitantly in all the cases. Two of the cases were traffic accidents.
Our study shows that MPA is found in DUID cases and reveals that NPS are used among drivers, and also proven in blood from drivers involved in traffic accidents. More studies are requested regarding the pharmacological and toxicological effects of MPA and other NPS. This is the first paper that describes a method for analysing and quantifying MPA in whole blood samples, and no DUID cases with MPA have previously been reported. Our study has shown that the prevalence of MPA in DUID cases in Norway was 0.8% in a time period of 12 weeks. Other potent psychoactive drugs were detected in all the cases, making it difficult to identify the impairing effects of MPA.

P188. VOLUPTUARY USE OF KETAMINE: COMPARISON AMONG SINGLE-PHASE TEST, AUTOMATED SCREENING AND DONFIRMATION TESTO IN GC/MS FOR THE TREACEABILITY OF METABOOLITES ON URINE.

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The use of ketamine, for non-medical purpose, results widespread also in Italy. This trend is documented through many evidences emerging from our researches on internet and social media. Nevertheless this drug is not included in the list of substances generally searched in the clinical analysis and forensic analysis by institutional centers, charged of the responsibility to realize the execution of toxicological analysis based on the article 187 of The New Italian Highway Code. In our previous works we already remarked the relevant presence of ketamine on casualty patients involved in car accidents in Rome. In this work, following our precedent works, we tested the reliability of the single-phase test, "52nd annual meeting of TIAFT 2014" and the automated method "17° Convegno Nazionale SITOX 2015" conducted at the Hospital Authority San Camillo-Forlanini of Rome, verifying with a further investigation, using gas chromatography-mass spectrometry (GC/MS), those samples already checked and resulted positive by screening. To strengthen the confirmation test, we did collaborate with the clinical laboratory of analytical toxicology of Polyclinic San Matteo of Pavia. In our previous works, the screening analysis were performed by a single-phase test (with a cut-off settled at 1000 ng/ml), and an automated immunoenzymatic assay, using "Indiko" instrument (cut-off settled at 330 ng/ml). The confirmation tests carried out in the present work has been realized by gas chromatography-mass spectrometry (GC/MS). The results from the first work, realized with a single-phase test, highlighted ten positive samples out of 294 samples, three of these were positive only to ketamine. In the second work the automated instrument confirmed only six out of ten previous positive samples, meanwhile the instrument found further four positive samples, considered negative by the single-phase test. In this work we confirmed by GC/MS the presence of ketamine only in seven samples out of fourteen resulted positive from both screening analysis. Three samples out of seven confirmed in GC/MS were positive only to ketamine. Following the law indications, nowadays is not foreseen to proceed to identify ketamine, using screening procedures and consequently with confirmation test. This operational limit does not make the authorities able to apply the penalties expected for road laws violations, for those people resulting positive to ketamine. From this work emerges the necessity to renovate and make more dynamic the operative protocols regarding the substances spectrum to analyse by screening test, referring those that seems to be the most widespread, based on epidemiological data. The automation, extended to an increasing number of detectable drugs, is essential to guarantee the reliability of toxicological tests, especially to medico-legal significance. Notwithstanding, this results highlight the absolutely necessity of the execution of the confirmation test, successively to screening analysis, where the cut-off not yet completely standardized proves a low reliability in comparison with toxicological dosages consolidated from a long time.

P189. EVALUATION OF THE HIP-FLASK DEFENCE BY USE OF ETHYL GLUCURONIDE IN BLOOD

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The hip-flask defence (i.e. claiming ethanol intake after an incident) is difficult to exclude from ethanol analyses, as these may show decreasing concentrations shortly after intake of alcohol. The non-oxidative metabolites of ethanol, ethyl glucuronide (EtG) and ethyl sulphate (EtS) have a different kinetic profile, with peak concentrations in blood around 4 hours after intake.
The aim of this study was to describe the method for using EtG in this purpose and to report cases in which this method is used.
Previously published studies are summarised. Also, in expert witnesses in cases where the hip-flask defence is claimed, EtG and EtS are analysed in selected cases using a previously published and fully validated ultra-performance liquid chromatography-tandem mass spectrometry method. Such cases are reported.
So far, about 70 healthy volunteers have been included in different kinetic studies, demonstrating maximal individual concentrations of EtG below 0.5 mg/L after 1 hour, below 1 mg/L after 2 hours and somewhat above 1 mg/L 4 hours after a moderate alcohol intake (up to 80 grams of ethanol). Higher intakes are unfortunately not studied in controlled experiments, so concentrations of EtG after larger doses have to be extrapolated assuming a linear relationship. After large and repeated alcohol ingestions, the limited research performed so far indicate that EtG concentrations return to very low levels about 24 hours after end of drinking. One known exception is patients suffering from kidney failure.
We report 12 cases, where the suspect claimed no alcohol intake before driving, only intake after driving. EtG and EtS were analysed as a part of expert witnesses. In all these cases, ethanol showed decreasing values in two consecutive samples (separated by approximately 30 minutes). Median 102 minutes (range 40-175) passed between the claimed intake after driving and the first blood sample. Median concentration of ethanol was 2.35 g/kg (range 0.86-3.15) in the first blood sample. The median EtG concentration in the first sample was 4.13 mg/L (range 2.0-7.4). Therefore, in all cases, the levels of EtG were substantially higher than what would be expected only about 1-2 hours after a very recent alcohol intake, also when taken into account that the detected levels of ethanol indicated intakes about twice the ones investigated in controlled, kinetic studies. The median EtG concentration in the second sample was 4.34 mg/L (range 2.1-7.2). One case showed an increase in EtG concentrations of 15% from first to second sample, in the rest of the cases, EtG concentrations was relatively stable. This also indicates that the high EtG concentrations were not caused by a fast formation after a recent intake, as this would require rapidly

increasing concentrations. EtS concentrations showed somewhat lower concentrations.
In conclusion, EtG and EtS in blood could be a helpful tool in assessment of the hip-flask defence, in cases where the detected ethanol is explained to be caused solely by a single intake after driving. Measurements in two consecutive samples make the interpretation more accurate.

P190. DETERMINATION OF 11-NOR-9-CARBOXY-TETRAHYDROCANNABINOL IN- NOR HAIR BY GAS CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY IN ORDER TO PROVE CANNABIS USE

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The scientific literature of '90 about hair analysis and Cannabis was focused on the detection of Δ9-tetrahydrocannabinol (THC), Cannabinol and Cannabidiol. Unfortunately, these substances are incorporated in hair not only after cannabis is smoked but also by passive exposure. So, an unequivocal proof of cannabis consumption is the detection of 11-nor-Δ9-carboxy- tetrahydrocannabinol (THCCOOH), the main metabolite of THC. This analyte is incorporated into the hair at very slow concentration and the cut-off recommended was set at 0.2 pg/mg . A very sensitive and robust method has to be developed to assure accurate results at this level of concentration.
The present study describes a gas chromatography tandem mass spectrometry-negative ion chemical ionization assay (GC-MS/MS-NCI) for the analysis of THCCOOH in hair. The method was validated and applied to hair specimens collected from subject that had to be submitted to hair analysis for different purposes (driving licences, WDT, follow up).
20-50 mg of hair sample, previously washed and pulverized was dissolved in 0.5 ml of NaOH 5M (75°C, 1h). Δ9-tetrahydrocannabinol (THC) was isolated with a first liquid/liquid extraction with n-hexane: ethylacetate 9:1 and analysed by GC-MS. The residue was extracted once again, after acidification, with the same mixture in order to isolate the THC metabolite. The extract was derivatized with PFPA and PFPOH and analysed by GC-MS/MS-NCI mode (Agilent 7000C).The method was validated and applied both to old samples which resulted positive for THC and to new specimens of routine analysis.
This method shows a good linearity in the range 0.05 - 5 pg/mg, (R^2=0.9998). Intraday, precision and accuracy were better than 10 and 15% respectively. The limit of quantification was 0.04 pg/mg and limit of detection 0.02 pg/mg. The concentration of THC and THCCOOH in the real samples ranged from 0.052-2.2 ng/mg and 0.0495 - 23.84 pg/mg, respectively. The 78% of samples positive for THC were also positive for THCCOOH, while in the 16% of cases THCCOOH was absent. A metabolite concentration under the cut-off was detectable in the 6% of THC positive samples. A relationship between THC/THCCOOH ratio and purpose of hair analysis (driving licence, WDT, follow up) was observed and could be related to different pattern of drug consumption.
The method proposed shows the right level of sensitivity and robustness to achieve and also exceed the concentration level requested and to be applied in routine analysis. Results coming from real samples underline the usefulness of the determination of THCCOOH in hair in order to obtain the proper interpretation of analytical data.

P191. IDENTIFICATION OF THE MAIN METABOLITES OF AB-CHMINACA IN HUMAN URINE USING UHPLC/MS/MS AND GC/MS TECHNIQUES

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AB-CHMINACA (N-[(1S)-1-(aminocarbonyl)-2-methylpropyl]-1-(cyclohexylmethyl)-1H-indazole-3-carboxamide) became a controlled substance in Hungary on July 19th, 2014 and on January 1th, 2015 it was transferred to the list of drugs-of-abuse. It was the most widespread synthetic cannabinoid in Hungary in 2014. Although it can be detected in human urine after consumption in most of the cases its concentration is very low and its metabolism were unknown at that time. Therefore we began to look after possible metabolites which can be present at higher concentrations to improve the efficiency and reliability of the screening and confirmatory methods. A presumed metabolite was first detected by HPLC-DAD.
The aim of this study was to find the possible metabolites of AB-CHMINACA in human urine and to prove their chemical structure through mass spectra and derivatization techniques using Shimadzu UHPLC/MS/MS and GC/MS instrumentation.
Urine samples of AB-CHMINACA abusers were selected. Two different sample preparations have been performed. One was done with acidic hydrolysis, followed by solid phase extraction on BakerBond narc-1 cartridges. Aliquots of the extracts were analyzed using a Shimadzu UHPLC-DAD-MS/MS in scan mode to determine the molecular mass of the compound seen on HPLC-DAD. Then UHPLC/MS/MS was applied in product ion scan and finally in multiple reaction monitoring (MRM) mode. The column was Kinetex C18 (100x3.0 mm, 2.6 µm), the eluents were acetonitrile with 0.1% formic acid and formic acid buffer pH 4. Other part of these extracts were derivatized with iodomethane and iodomethane-D3 and analysed by GC/MS (capillary column: Agilent VF-DA 12x0.20, linear velocity and splitless mode, acquisition mode: scan). The second way of sample preparation was performed in parallels by solid phase extraction on Agilent Bond Elut Plexa cartridges with and without enzymatic hydrolysis using Escherichia coli (IX-A) β-glucuronidase. These extracts were analysed using UHPLC/MS/MS in MRM mode.
The following ion fragments were found and set in MRM mode on the UHPLC/MS/MS corresponding to presumed metabolites: 358>145, 241, 97 (carboxylate metabolite); 373>145, 257, 95 (hydroxylated metabolites); 374>145, 257, 95 (hydroxylated carboxylate metabolites). The last two MRM transitions provided two significant peaks with different retention times, which refers to the existence of structural isomers. Hydrolysed and non-hydrolysed samples

were compared and the degree of glucuronidation calculated. Applying GC/MS analysis derivatives of two main presumptive metabolites have been identified. One is probably the carboxylate metabolite (methyl derivative: m/z 371 (M^{*+}), 312, 241; trideuteromethyl derivative: m/z 374 (M^{*+}), 312, 241). The other one probably arises from the carboxylate metabolite and comprises hydroxyl group on the cyclohexyl ring (stereochemically not characterized; bis-methyl derivative: m/z 401 (M^{*+}), 342, 271; bis-trideuteromethyl derivative: m/z 407 (M^{*+}), 345, 274).

P192. GHB-GLUCURONID - PILOT STUDY INVESTIGATING CLEAVABILITY, STABILITY, AND ENDOGENOUS CONCENTRATIONS IN HUMAN URINE SAMPLES

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Recently, Petersen et al. (2013) showed that gamma-hydroxybutyric acid (GHB) is metabolized by conjugation to glucuronic acid, in addition to the pathway by the Krebs cycle. At present, there are few data on endogenous GHB-glucuronide (GHB-gluc) concentrations in human specimens. In 50 urine samples, Petersen et al. found 0.11 to 5.0mg/L GHB-gluc (mean=1.3mg/L). Mehling et al. (2014) found up to 0.042mg/L GHB-gluc in seven serum samples. GHB-gluc was quantified directly by LC/MS-MS methods in both studies. While Petersen et al. proposed a good stability for GHB-gluc, the authors of the latter study pointed to a possible extreme instability of this analyte.
In our pilot study, we investigated the following issues: a) Can GHB-glucuronide be cleaved by β-glucuronidase? b) How are the ratios of free GHB to bound GHB? c) How stable is GHB-gluc under routine conditions?
Cleavage of GHB-glucuronide was performed by the addition of 20µL β-glucuronidase-solution (Roche, Mannheim, Germany) at 42°C over 75 minutes (pH 6.0-6.5). The free GHB was quantified after liquid/liquid extraction and derivatization with BSTFA + 1%TMCS (N,O-bis(Trimethylsilyl) trifluoracetamide with Trimethylchlorosilane) via GC/MS in SIM mode (LOD=0.1mg/L). Determination of the concentration of GHB-glucuronide was done by calculating the difference between the concentration of GHB with and without glucuronide cleavage in the same sample. Endogenous GHB-gluc concentrations and free-to-bound ratios were determined in 31 fresh human urine samples. The stability of GHB-gluc was investigated using six urine samples stored at -20°C and 4-8°C over 21 days (both with and without preservation (1% NaF)).
In fresh urine samples (n=31) we found <0.1-0.92mg/L of free GHB (mean=0.30±0.23mg/L; median=0.19mg/L), 0.21-1.27mg/L of total GHB (GHB-tot) (mean=0.63±0.34mg/L; median=0.54mg/L), and calculated 0.04-0.69mg/L of GHB-gluc (mean=0.33±0.17mg/L; median=0.34mg/L). The proportion of GHB-gluc in the concentration of GHB-tot was 27%-82%, the mean of free-to-bound ratios in fresh urine samples was 1.09±0.87 (range=0.22-4.3; median=0.97, n=31). Therefore, there was large inter-individual variation. After storage of six urine samples for three weeks,. the absolute amount of GHB-gluc remained stable in four out of six urine samples. In two out of six specimens, the concentration of GHB-gluc decreased by more than 20%. In these two cases, the decrease was independent of storage conditions and occurred at 4-8°C and -20°C in samples with and without preservation. However, the influence of temperature and sample preservation could not be interpreted clearly in this small selection.
Analogue to other glucuronides, the glucuronide of GHB can also be cleaved by β-glucuronidase. First results may suggest that an individual sample's properties have more influence on GHB-gluc stability than storage conditions. In this study, concentrations of urine GHB-gluc were below 1mg/L and ratios of free-to-bound GHB were above 0.22, but both were quite variable. Therefore, it is not clear whether GHB-gluc can be applied as a future biomarker to discriminate endogenous from exogenous GHB.

P193. PROFILE OF COCAINE CONSUMED IN BOGOTÁ (COLOMBIA) AND SURROUNDING AREAS AT PRESENT.

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The use of cocaine in Bogotá and surrounding areas is present in form of salt, consumed sniffed nasally mainly 'perico' and in cocaine paste smoked pulmonary route 'basuco', according to the National Study of Psychoactive Substances in Colombia 2013 which reports that 0.49% of Bogotá's population states to have consumed cocaine during the last year. According to retrospective analysis of data generated from our laboratory, the higher prevalence of seizures belongs to cocaine paste followed by salt form. The low price and its easy access allows the user to get it without difficulty; however, this material and refining cocaine byproduct is not exempt, like salt, of cutting substances adding that can increase dealers profits and threatening users lives.
The AIM of this study is to show cocaine profiles that are seized from users and dealers in Bogotá and surrounding areas through the National Police of Colombia. Moreover, this research intends to show the clear relationship between cutting substances and cocaine seized types; as well as the risks associated to the consumption of these substances which users are exposed, not counting cocaine consumption threaten itself.
Each sample was pulverized, homogenized and treated with ethanol leave theme in ultrasound bath for 15 minutes, then the solution was analyzed by GC-MS using tetracosane as internal standard. Gradient elution was performed by HP-5MS capillary column Split injection mode, the ionization of the sample was performed by electron impact at 70 eV EI in scan mode (35 and 450 amu). The GC-MS technique is validated and certified for analyzing cocaine and cutting agents. For statistical analysis, 800 random samples of seized cocaine were taken during 2013, 2014 and early 2015. Subsequently, the cutting substances which were on cocaine were determined, along with their relationship depending on the presentation.
The main result analysis showed that from the total population of seized cocaine and examined, 76.8% belongs to paste and 23.2% are in salt form. Within the base paste samples, drugs like caffeine and phenacetin (51.2% and

46.8% respectively) were frequently found and levamisole (5.0%) and lidocaine (4.7 %) in a lesser percentage. In contrast, it was found that salt form cocaine mostly present levamisole as cutting agent (80.6%), followed by caffeine and lidocaine (22.6% and 21.5%, respectively) and lower percentage, phenacetin (8.6%). In most samples treated were two or more of the aforementioned substances as well as few other substances, such as aminopyrine, diltiazem, guaifenesin and/or dimethylterephthalate. We also found that 21.3% of all samples did not present none of the above cutting substances.
In summary, seized and analyzed cocaine is mainly represented by cocaine paste (76.8%) found mainly cut with caffeine and phenacetin. Approximately one quarter of cocaine seizures are in salt form and are mostly cut with levamisole (80%).Furthermore In Colombia cocaine without quite toxic adulterants, as those already mentioned (21.3%), are still found.

P194. **INTENSIFIED SCREENING PROGRAM (ISP) FOR CONCOMITANT DRUG USE IN PATIENTS ON OPIOID MAINTENANCE THERAPY (MT) BY HAIR TESTING**

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Concomitant use of both illicit and pharmaceutical drugs, as well as of alcohol, is a serious problem in clinical practice of MT since it interferes with actions of the substitute, thus jeopardizing patient's health and MT success. Closer data on frequency and type of concomitant compounds are sparse, especially because of insufficient screening in the physician's office, normally by using diagnostic dipsticks on unattendedly obtained urine samples. Following serious and even fatal intoxications of patients and their children, an iSP for concomitant drug abuse in MT was established in Bremen in 2012.
Here, preliminary data obtained through end of 2014 are reported.
The iSP in Bremen consists of structured anamnestic and advisory interviews with addiction experts and of hair or urine analysis for illicit and pharmaceutical drugs and for ethyl glucuronide. The respective information is communicated with the patient, the physician responsible for MT, and with a quality assurance commission which continuously supervises MT. Hair specimen are taken according to SOHT standards, urine samples are obtained under close supervision, qualitative and quantitative analysis is performed by CEDIA, DRI, GC/MS, and LC/MS/MS. The present evaluation reports on patients who were believed to be devoid of concomitant drug use.
96 subjects (71 male, majority 30-50 y) on MT (32 methadone, 55 levomethadone, 9 bu¬prenor-phine; 34 take-home setting) were included. Only 14 (15 %) were free of concomitant drug detection. In 40 (42 %) cases one additional concomitant drug was detected, in 21 (22 %) patients two compounds and in 21 (22%) three or more compounds. Alcohol, benzodiazepines, cannabis and diacetyl¬morphine (in order of frequency) were the prevailing compounds, stimulating drugs were occasionally detected. In 7 patients with proven concomitant drug abuse, repetition of the iSP was performed after approx. one year. Five of these 7 patients showed freedom or at least reduction in number of concomitant drugs. Interestingly, there was a high correlation (60 %) between information on concomitant abuse from interviews and analytical results.
An iSP provides much more information on concomitant drug abuse in MT patients than the common screening practice. In the present project iSP increased the risk awareness in all parties and may result in higher safety for patients and their relatives.

P195. **DETERMINATION OF GHB LEVELS IN BREAST MILK AND CORRELATION WITH BLOOD CONCENTRATIONS.**

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Among recreational drugs of abuse Ð–hydroxybutyrate (GHB) is a particularly troublesome substance, because of its ready availability, low cost, and risk of adverse interaction with other sedative drugs. The sodium salt of GHB is registered as a therapeutic agent (Xyrem®), approved in some countries for the treatment of narcolepsy-associated cataplexy and (Alcover®) is an adjuvant medication for detoxification and withdrawal in alcoholics.
The first aim of this study is to determine the endogenous levels of GHB in breast milk and blood samples of 12 breastfeeding women and determining if there is a correlation between blood and breast milk GHB concentrations. Secondly, GHB levels will be determined in breast milk and blood samples of a 32 year-old breast-feeding narcoleptic female, who was prescribed sodium oxybate (4.5 g twice daily).
Blood and breast milk samples were collected from 12 breastfeeding women (mean age 29.5 ±3.9 years) between the second and the fourth month of breastfeeding. For each woman 2 blood and 2 breast milk samples were collected with an interval of one week (total 24 blood and 24 breast milk specimens).
Blood and breast milk samples of a 32 year-old nursing mother, who was on sodium oxybate treatment, were collected. On the 10th week of lactation only breast milk samples were taken (total: 10 breast milk samples, 5 from each breast) at 0.5, 1, 3, 4 and 5 hours following a 4.5g GHB dose, whereas on the 11th week of lactation blood and breast milk samples were simultaneously collected at the same intervals of time after a 4.5g GHB dose. The GHB concentrations were measured by GC-MS after liquid-liquid extraction according to a previously published method, which was readapted to the conditions of the present study and fully validated.
The endogenous GHB levels in blood ranged from 0 to 2.51mg/L, whereas the endogenous GHB levels in breast milk

ranged from 0 to 1.76mg/L showing a significant statistical correlation (p<0.001). The GHB blood concentration 1 hour after drug administration was 80 mg/L and it steadily decreased to reach a level of 1.2 mg/L, 5 hours after the medication intake. GHB blood concentration of 57 mg/L was found 0.5 hour after dosing. The GHB concentrations found in breast milk were substantially lower following the same pattern as for the blood (p<0.001), with the highest concentration being 56 mg/L, 1 hour after sodium oxybate administration and the lowest 0.9 mg/L, 5 hours after the medication's intake.
This study highlights how breast milk may represent a valid matrix for the determination of endogenous and exogenous GHB levels. A good correlation between blood and breast milk concentrations was found (p<0.001). We would recommend, taking into consideration these preliminary data, that nursing mothers under sodium oxybate treatment should breastfeed at least 5 hours after the last GHB administration. However, further studies are necessary in order to confirm these findings.

P196. A GC-MS METHOD FOR DETECTION AND QUANTIFICATION OF CATHINE, CATHINONE, METHCATHINONE AND EPHEDRINE IN ORAL FLUID

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The stimulating herbal drug kath and its analog methcathinone are commonly substances of abuse in most countries. However, information on their detectability in oral fluid is limited.
The aim of this work was to develop and validate a GC-MS method for the detection and quantification of cathine, cathinone, methcathinone and ephedrine in oral fluid samples.
Fresh oral fluid specimens for calibrators and quality control samples were donated by the staff personnel. Authentic specimens were collected from khat and methcationone users. Oral fluid were collected with a cotton pad collection device, centrifuged and then preserved by adding 50-mg citric acid to 1.0 mL specimen. The analytes and internal standard (amphetamine-D5) were extracted from 0.5 mL oral fluid spiked at concentrations in the range 20–2000 ng/mL, by liquid extraction with ethyl acetate; the dried extracts were derivatized with heptafluorobutyric anhydride (HFBA) at 70 °C for 30 min. GC-MS was used for detection. The MS was run in selected ion monitor (SIM) mode. The ions m/z 117, 240 and 330 (cathine), m/z 77, 105 and 240 (cathinone), m/z 105, 210 and 254 (methcathinone), m/z 210, 254 and 344 (ephedrine), and m/z 244 and 337 (amphetamine-d5) were used as quantifier or qualifier ions. The GC-column was VF-5ms (30 m × 0.25 mm i.d., 0.25 mm film thickness).
The calibration cure was linear from 20 to 2000 ng/mL for all analytes. Intra- and inter-assay imprecision were lower than 10% (RSD), and the bias of the assay was lower than ±15% for all the compounds, estimated as % recovery error. Limits of quantification were 20 ng/mL for all analytes. The method was used to detect and quantify cathine, cathinone, methcathinone and ephedrine in authentic oral fluid specimens.
The method was validated and successfully applied to saliva specimens collected from khat and methcathinone users.

P197. EVALUATION OF PROLONGED EXPOSURE TO MICROCYSTIN IN MICE

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Cyanobacterial blooms in natural waters, causing serious water pollution and public health hazard to humans and livestock, have become a worldwide problem. Species of the genus Microcystis, are known for their potential ability to synthesize toxins, mainly microcystins (MC). The effects of prolonged exposure to low dosis of MCs on health are not yet sufficiently understood and this type of poisoning is often undiagnosed.
The objective of this work was to explore different tissue damages generated by oral prolonged exposure to 50 to 100 µg MC-LR/kg in a murine model
MC-LR was purified from natural blooms of M. aeruginosa collected at the Rio de la Plata basin, Argentina. The purification was performed with semi-preparative HPLC HP 1100 with diode array detector system. The toxin was tested by the HPLC–MS method. Thiobarbituric acid reactive substances (TBARS), superoxide dismutase activity (SOD) and glutathione profile were study in different tissue. In addition, the determination of MC-LR levels in liver, kidney, plasma, urine and feces of treated mice was carried out.
We found alterations in TBARS, SOD activity and glutathione profile in liver and intestine of mice exposed to both doses of MC-LR tested in this work. In addition the presence of MC-LR was detected in both organs. Also, we found 3.6 ± 0.6% and 15.3 ± 1.6% of hepatic steatosis and intraepithelial lymphocytes decrease of 28.7 ± 5.0% and 44.2 ± 8.7% in intestine of 50 and 100µg MC-LR/kg treated animals respectively. This result could have important implications in mucosal immunity, since intraepithelial lymphocytes are the principal effectors of this system.
Our results indicate that intermittent and prolonged oral exposure at doses as low as 50 µg MC-LR/kg each 48 hs generates damage not only in liver but also in intestine. This finding is interesting taking into account that a dose of 40 µg MC-LR/kg (derived from experiments of daily administration) is a NOAEL actually accepted.

Poster abstracts

P198. **STUDY OF INHALED HALLUCINOGENS COMING FROM SOUTH AMERICAN PLANTS ON HAIR**

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Inhalation of hallucinogenic powders is an ancient practice with very peculiar socio-cultural and religious connotations that persists even today, both in the northwest Argentina and elsewhere in South America. A major source of hallucinogenic substances for inhalation are Anadenanthera spp., trees possessing psychoactive dimethyltryptamines. The aim of this work was to detect this hallucinogen or related compound in pericranial hair of two deceased natives.
Hair samples of 2 individuals died over a century ago were processed. The cuticle damage was firstly microscopically tested and then successively washings with dichloromethane and water were carried out at 37°C. Washing liquids were kept for futher analysis. The sample digestion was performed with 0.1 N HCl at 50°C for 24 hours and then filtered. The extract was processed by SPE (Bond Elut C-18), taken up with 500 µL of methanol. The extracts were injected into a Shimadzu GC 17 -QP 5000 (GC-MS). HP-DB5 column (0.25 mm x 60 m). Carrier: 0.7 ml / min. Ti 50°C - 120°C grad.30°C / min and from 120°C to 250°C grad. 15°C/min. T inj. 260°C/min. Split ratio: 8, IS: trypatmine 1 ng/µl. External standard was used. LOD and LOQ were 0.1 ng/mg and 0.4 ng/mg.
Microscopic hair examination showed no cuticle damage. 5-methoxy dimetiltriptamine (5MeO-DMT) was detected in retention time (tr): 6.25 min (15.0 ng / mg hair) IS: tr 7.36 min. The washings showed no signals attributable to the 5MeO-DMT.
The 5MeO-DMT is a good indicator of consumption of hallucinogens from Anadanthera spp. Future studies are required to investigate other biomarkers for compounds consumption.

P199. **EXPERIMENTAL STUDY ON THERAPEUTIC USE OF CANNABINOIDS**

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The Cannabis, well-known for its psychoactive effects, is widely used for a multitude of pathological conditions. Since 2013 in the Italian market has been introduced the Nabiximols (Sativex®), a drug containing the two main active ingredients of cannabis: Ð9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD). This drug is used in our Country in the treatment of Multiple Sclerosis (MS) and it is administered via the oral mucosa, each puff consists of 100 µl containing 2.7 mg of Δ9-THC and 2.5 mg of CBD.
We analyzed urine and blood samples collected from a group of patients treated with Nabiximols in order to evaluate: cannabinoid concentrations in relation to the dose administered and the duration of treatment; the potentiality of this patent medicine to be used for drug habit; and if it is able to cause drive impairement.
The study was conducted on a sample group of patients affected by MS of both sexes (age: 49-61 years), treated with Nabiximols for short (28 days) or long term, which gave their informed consent. The protocol evaluated blood and urinary cannabinoid concentrations in relation to the dose administered and the duration of treatment. Urine samples were analyzed before starting treatment and after that, once a month for three months. The patients which gave their consent also for blood tests were undergone to analysis immediately before and at fixed intervals (15 min, 30 min and 60 min) after Nabiximols administration. The first urine analysis were performed by immunoassay Drug-Screen-THC (Nal Von Minden®), for the qualitative detection of THC-COOH (cut-off: 50 ng/ml). Blood samples (1 ml) were analyzed by liquid/liquid extraction with cicloexane, and the extract, derivatized by BSTFA + 1% TMCS, analyzed by GC/MS in SIM mode.
In order to exclude a prior cannabis exposure, the urine samples were analyzed before the starting of therapy with Nabiximols. All samples were negative for cannabinoids. The follow-up urine analysis were performed after 1, 2 and 3 months after starting therapy and all patients resulted positive, with values above 50 ng/ml. In blood, the Δ9-THC was detected in extremely small concentrations (ranged from not detected to 2.36 ng/ml).
It was reported a peak plasma concentration of 30 to 120 ng/ml within 3 min after termination of smoking a Cannabis cigarette (containing 13.0 mg of Δ9-THC). In our study concentrations more than 10 times smaller were observed. The results of our study allowed us to affirm that it is not possible to use this medicine for drug habit, to sale it in the gray market or to induce drive impairment. This statement was confermed by the low blood concentrations, the low Δ9-THC concentration in pharmaceutical formulation, the presence of CBD (Δ9-THC antagonist) and the route of administration.

P200. **BIOENERGETIC DISRUPTION IN HEPG2 CELLS BY ANTITUBERCULOUS DRUGS INDUCED CYTOTOXICITY**

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Anti-tuberculus(Anti-TB) -induced hepatotoxicity is a potential serious adverse effect of these drugs.
In this study, the effect of Anti-TB drugs; isoniazid (INH), rifampicin (RIF) and pyrazinamide (PZA) on HepG2 cells bioenergetics was investigated.
Intracellular ATP assay was investigated 4h, 24h and 48h post-exposures to different concentrations of the studied Anti-TB drugs. Then, the effects of tested drugs on mitochondrial membrane potential, mitochondrial complex I activity and cellular lactate production were assessed.
ATP assay showed that the tested drugs decreased the intracellular ATP levels in HepG2 cells in relation to their

concentrations and exposures durations. Estimated 24h IC50s were 0.5mM, 70mM and 84mM for RIF, INH and PZA respectively. Mitotracker green assay showed that all the tested drugs significantly decreased mitochondrial membrane potential in their estimated IC50s, 24h post-treatment(P-value=0.0005). IC50s the tested drugs were found to significantly decreased complex I activities, 24h post-treatment(P-value=0.0003).HepG2 lactate production was found to significantly increased with the tested Anti-TB drugs IC50s, 24h post-treatment (P-value=0.0008). RIF and INH in low concentrations were found to significantly increase the effect of each others on HepG2 mitochondrial complex I.
Anti-TB drugs RIF, INH and PZA significantly affect HepG2 bioenergetics in their toxic concentrations, RIF is the most effective. Their combination showed cumulative effect on the cells bioenergetics, which means that patients on these medications are more vulnerable to their toxicities with less significant over-ingestions.

P201. STUDY OF CANNABIS INDUCED WEIGHT LOSS

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Cannabis is one of the world's oldest cultivated plants. Currently, however cannabis cultivation and use is illegal or legally restricted around the globe. Despite constant official control, Cannabis cultivation and use has spread to every continent and nearly every nation. Cannabinoid components of cannabis are known to exert behavioral and psychotropic effects but also to possess therapeutic properties. The endocannabinoid system is one of the regulator of appetite. Endocannabinoids stimulate food intake through homeostatic and non-homeostatic mechanisms via the cannabinoid 1 (CB1) receptor. Leptin is an adipocyte-derived hormone that is secreted in correlation with total body lipid stores. Serum leptin levels are lowered by the loss of body fat mass that would accompany starvation and malnutrition. Leptin is a key adipokine involved in food intake regulation. This hormone affects multiple neural circuits involved in the control of energy balance.
This study is a trial to explain the cannabis induced weight loss by determination of the effect of smoking cannabis on (appetite control hormone) leptin concentration compared with healthy non smoker subjects.
9-carboxy-Tetrahydrocanabinol (THC) was detected in the urine of positive THC males comprising Group II (GII)and compared to those of control healthy male as volunteers with negative (THC) comprising group I (GI) of matched age and Body mass index (BMI). THC was analyzed qualitatively by using Gas Chromatography-Mass spectrometry (Agilent 6080N- capillary coloumn 30m- 1µ diameter). Serum 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. The method depended on a non-competitve assay in which the analyte to be measured was "sandwiched" between two antibodies.
Serum leptin concentrations were decreased in GII (4.7 ± 0.84 ng/ml) than in GI (6.47 ± 1.39 ng/ml) associated with decreased body mass index (BMI) in GII (21.91 ± 2.30) than in GI (22.1± 2.27).
cannabis addiction is associated with modulation of serum leptin concentration thus confirming the interactions between leptin and cannabis in food intake control that may be a target for obesity treatment.

P202. LEAD EXPOSURE RISK ASSESSMENT AMONG EGYPTIAN NEONATES USING NEW STRUCTURED QUESTIONNAIRE

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A child's lead burden begins before birth and increases in the first few years of life. CDC had changed the childhood lead screening from universal testing to target testing based on the risk of lead exposure. In Egypt, high cost of performing blood lead levels (BLLs) for all newly born can't be afforded. There is no yet implemented standardized lead testing program based on risk of lead exposure.
This study was conducted to design and evaluate the role of a lead risk questionnaire in predicting elevated BLLs in Egyptian neonates.
Structured questionnaire was created and conducted based on known environmental exposure and risk factors in Egypt. Blood samples of 400 neonates were measured for BLLs to indicate exposure biomarker. The significantly associated questions to high BLLs were plotted on a logistic regression form to order them for accountability of high BLLs.
Bottle feeding practice (OR, 2.2; 95%CI, 1.2-3.9) increases the risk of occurrence of lead toxicity to nearly four times more than non-bottle feeding practice (OR, 0.6; 95%CI, 0.5-0.8). Bottle feeding practice (ß= 1.387, p = 0.014) followed by postnatal age (>10days) (ß=0.94, p =0.032) were the most significant predictors of lead toxicity as they accounted for 17.7% of the variance of the neonatal BLLs.
It is recommended to apply this risk assessment questionnaire to all health care units to identify neonates and children at risk to high blood lead burden, however, it is highly advised to test the questionnaire in various setting to figure out how effective it is.

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P203. STUDY ON THE TOXICOKINETICS OF DIAZEPAM AND ITS(I AND IIPHASE) METABOLITES IN HAN PEOPLES IN CHINA

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Driving after a dose of diazepam was met in China. The pharmacokinetics of diazepam and its metabolites in Chinese was not reported, which was useful for the test and identification of drug driving. In this study, the pharmacokinetics of diazepam and its I and II phase metabolites in Chinese Han people was investigated.
To observe the pharmacokinetics and the detection time limit of diazepam and its metabolites in blood, urine, saliva of Han people in China;To investigate the relationship between time of last diazepam use and diazepam/ metabolite concentration ratios or metabolite/ metabolite concentration ratios, to establish a model for estimating time of last diazepam use.
Twenty eight participants from Taiyuan were given an oral dose of 5mg diazepam. Before the experiment and at 1h, 2h, 4h, 8h, 12h, 24h, 2d, 3d, 6d, 12d, 15d, blood and saliva, urine were collected for the determination of diazepam and its metabolites. Diazepam, nordiazepam, oxazepam, oxazepam glucuronide, temazepam glucuronide in specimens were extracted with solid phase extraction (SPE) and determined by HPLC-MS-MS qualitatively and quantitatively with a MRM model.
(1)The diazepam, nordiazepam, oxazepam, estazolam, oxazepam glucuronide, temazepam glucuronide could be detected simultaneously by a SPE- HPLC-MS-MS with a reasonable accuracy, precision, LODs, recoveries, linear range. (2)After an oral dose of 5mg diazepam, diazepam, nordazepam, oxazepam, oxazepam glucuronide and temazepam glucuronide all could be detected in blood and urine. Except for oxazepam, the others could be detected in urine. The detection window ranged from less than an hour to more than 15d. (3)The pharmacokinetics of diazepam in blood met a two compartment model, t1/2k01, t1/2k12, t1/2k10 respectively were 0.3h±0.4h, 9.9h±13.6h and 37.2h±23.4h; The pharmacokinetics of nordazepam in the blood, urine and saliva met an one-compartment model, t1/2k01, t1/2k10 respectively were 38.4h±26.9h and 239.1h±289.4h; The pharmacokinetics of oxazepam glucuronide and temazepam glucuronide in the urine met an one-compartment model, t1/2k01, t1/2k10 respectively were 35.3h±32.5h, 234.0h±265.1h and 3.9h±5.2h,7029.4h±32137.7h. Oxazepam were detected in a lower concentration in 15d.(4) By blood, urine or saliva diazepam/nordiazepam concertrion ratios, urine a model for estimating time of last diazepam use was established, and an equation was developed to predict elapsed time of diazepam use. Especially the model was developed respectively from male or female blood diazepam/nordazepam concertrion ratios could provided an equation to predict elapsed time with a maximum error value less than 20% .
A simultaneous analysis of diazepam and its metabolites in biological samples by SPE-HPLC-MS-MS was established. The pharmacokinetics model of diazepam and its metabolites in human were established. It was firstly reported that the pharmacokinetics of diazepam and its metabolites(I and II phase) in the blood, saliva, and urine of Chinese people, which met a two and an one compartment model except for oxazepam. A preliminary model for estimating time of last diazepam use was established by blood or urine diazepam/nordiazepam concertrion ratios.

P204. GASCHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) DETERMINATION OF QUETIAPINE IN TWO NON-FATAL POISONONING: DIAGNOSIS OF INTOXICATION AND DRUG ELIMINATION MONITORING

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Quetiapine is a benzazepinic derivate and part of the family of atypical antipsychotics. Is currently marketed to treat schizophrenia and prescribed in association with other drugs acting in the CNS. Case 1: woman, 59 years old, admitted to Emergency after ingestion of 1,4 gr of quetiapine (Seroquel 25 mg, 56 tablets). Case 2: woman, 54 years old, admitted to Emergency after ingestion of an unknown quantity of quetiapine. In both cases the time elapsed between assumption and hospitalization was unknown and each patient has taken quetiapine for suicidal purposes. Blood, urine and gastric lavage were collected for each patient and sent to our laboratory. The collection continues until quetiapine's elimination from blood. Evaluate quetiapine's concentrations in biological samples for control therapy efficacy (decrease of serum concentrations and elimination through gastric lavage), compare pharmacokinetics of elimination in acute intoxications due to differents pharmaceutical formulations (immediate release in case 1, immediate and prolonged release in case 2). Serum, urine and gastric lavage were added of internal standard (Mianserin 500 ng/ml), buffered to pH 9 with borate 0,05 M and then extracted with 4 mL of chlorobutane. After separation and evaporation of organic phase, dry residue was reconstituted with MSTFA and derivatized at 75°C for 25 minutes.The analysis were performed with a Varian Saturn 4000: the carrier gas was helium and a DB-5 MS UI (30 m x 0,25 mm, 0,25 µm film thickness) capillary column was used for analyte's separation. The ion trap mass spectrometer operated in SIS mode, ions monitored were 193, 264, 192 for mianserin and 210, 239, 322 for quetiapine. Calibration curves were prepared by spiking drug-free serum with quetiapine at six levels, and extracting with the above procedure. The analytical method results sensitive (LOD = 10 ng/mL, LOQ 25 ng/ml) and was evaluated for specificity, LOD (S/N>3), LOQ (S/N>10), linearity (regression coefficient of 0,998 from LOQ to 500 ng/mL), precision, accuracy, repeatability. The results above the calibration range have been obtained from diluted samples, normalized 1:2, 1:5, 1:10 ratio.The two cases reported are very different as regards the amount of quetiapine taken, the type of pharmaceutical form (immediate release in case 1 and immediate and prolonged release in case 2), concentrations detected (peak serum of 865,6 ng/ml in case 1 and 9470 ng/mL in case 2) and the time of observation. In the first case quetiapine decreases quickly and was below therapeutic interval 1 day

after admission; in second case quetiapine reaches therapeutic range the seventh day of hospedalization: initially concentration was 5132 ng/ml, than 804 ng/ml after 12 hours, but after 24 and 36 hours increases to 5504 and 9470 ng/ml respectively. Finally it decreases following the same trend as in case 1. The results underline the different course in acute poisoning from quetiapine: the intake of the immediate release pharmaceutical form follows a first-order kinetics, while the assumption of different pharmaceutical forms (immediate and prolonged release) can lead to delayed concentration peak in blood that can be determined only extending the sample collection times.

P205. DISTRIBUTION OF 6-ACETYLMORPHINE, MORPHINE, AND CODEINE IN UNCUT HAIR, CUT HAIR AFTER HEROIN WITHDRAWAL

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Hair grows approximately 1 cm per month and can therefore reflect a retrospective timeline of drug exposure. Segmental hair analysis has been applied in workplace testing, the licensing of drivers, clinical compliance control and a number of forensic issues (e.g., drug abstinence control, the evaluation of drug-induced diminished responsibility and drug facilitated crimes). However, the variability in the growth rate of human head hair and the inconsistent collection of hair significantly affects the interpretation of results from the segmental analysis of hair. Until now, there was a lack of information on the decline of the concentration of drugs in hair after abstinence.
The objective of this study was to investigate the distribution of 6-acetylmorphine, morphine, and codeine in uncut hair (The subject has let it grow after abstinence began), cut hair (The subject had shaved his head one week after abstinence began) after heroin withdrawal.
Hair samples were obtained from 69 subjects with different heroin withdrawal times. All of the hair samples were cut into 1-cm segments for analysis. The hair segments were pulverised in a freeze mill. Twenty milligrams of powered hair was sonicated in 1 ml of borate buffer, pH 9.2, for 30 min. Then the analyte was extracted with 2 ml chloroform:isopropanol (9:1, v/v). The analysis was performed by using LC-MS/MS operating in MRM mode.
The LODs and LLOQs of developed LC-MS/MS method ranged from 0.01 to 0.02 ng/mg and from 0.02 to 0.05 ng/mg for the different opiates, respectively. For 43 uncut hair specimens, the mean elimination half-lives of 6-acetylmorphine, morphine, and codeine were determined to be 1.10±0.39 months, 1.68±0.44 months, and 1.52±0.45 months, respectively. For 26 cut hair, 6-acetylmorphine was detectable 17 samples (ranging from 0.4 to 4.4 ng/mg, mean = 1.4 ng/mg). However, only three samples contained detectable morphine.
Concentrations of opiates in cut hair were significantly lower than that in uncut hair. The time of the last haircut is important in the interpretation of results.

P206. EFFECTS OF PARAOXON AND OXIME K048 ON ACHE ACTIVITY AND PRIMARY DNA DAMAGE IN A549 AND HACAT CELL LINES IN VITRO

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Organophosphorus (OP) compound poisoning is an important worldwide clinical and public health problem. Some nerve warfare agents have relatively recently been used in terrorist attacks, while pesticide poisonings represent the leading cause of poisonings reported globally. OPs act primarily as neurotoxins due to the irreversible inhibition of acetylcholinesterase (AChE), but there is increasing evidence for secondary mechanisms of their toxicity. Oximes act as an essential part of antidotal therapy by ensuring the recovery of inhibited AChE. Our recent in vitro and in vivo studies have drawn attention to oxime K048 as a very potent reactivator of the nerve agent tabun- and pesticide-inhibited AChE.
This study evaluated OP toxicity at molecular level and its potential alleviation by the use of an oxime. For this purpose, as it is one of the most potent AChE-inhibiting compounds available, we selected paraoxon as a model OP and estimated its ability to induce primary DNA damage in two human cell lines known for non-neuromuscular AChE expression. By applying the K048, we tested its ability to extenuate the effects of paraoxon.
Lung adenocarcinoma epithelial cell line A549 and keratinocyte cell line HaCaT were cultivated in RPMI growth medium supplemented with glutamine, heat-inactivated fetal bovine serum, and antibiotics. Prior to treatment, cells were seeded in 6-well plates (3x105 cells/mL). The tested concentrations of paraoxon (1 μM), and K048 oxime (0.02 mM) were determined in a preliminary experiment on erythrocytes. For the genotoxicity testing, we applied the same study design in both cell lines: control group, cells treated with paraoxon (10 min), cells treated with K048 (30 min), cells treated 10 min with paraoxon followed by a 30-min treatment with K048 oxime. After the treatments, cells were used for the preparation of agarose microgels according to standard protocol for the alkaline comet assay. Slides were analyzed under fluorescent microscope using the Comet Assay IV analysis system. The level of DNA damage was evaluated based on comet tail length, tail intensity, and tail moment. AChE activity measurements were performed by using the standardized spectrophotometric Ellman method.
At the concentration tested, K048 did not induce a significant increase of primary DNA damage as compared to the negative control. In contrast, treatment with paraoxon, in spite of the relatively short exposure time, resulted in measurable genotoxicity both on A549 and HaCaT cells. At the same concentration, paraoxon caused a 93% erythrocyte AChE inhibition in our preliminary experiment. The most important observation was the lowering of primary DNA damage in cells treated with both compounds. In both cell lines, when applied after paraoxon, K048 caused a

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significant decrease of all comet assay parameters as compared to single paraoxon treatment. Furthermore, K048 was shown to reactivate about 87% of paraoxon-inhibited erythrocyte AChE.
High reactivation potency and low genotoxicity of K048 oxime at the concentration tested suggest its favorable properties, which are important for a compound intended for use as an antidote.

P207. RITUALISTIC USE OF AYAHUASCA VERSUS STREET USE OF SIMILAR SUBSTANCES SEIZED BY THE POLICE: A KEY FACTOR INVOLVED IN THE POTENTIAL FOR INTOXICATIONS AND OVERDOSE?

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Ayahuasca (also spelled ayawaska), is used to refer to decoctions of the liana Banisteriopsis caapi plus Psychotria viridis, and its ritualistic use is becoming a global phenomenon. This beverage contains a combination of monoamine oxidase inhibitors (β-carbolines, such as harmine, harmaline and tetrahydroharmine) and the alkaloid N,N-dimethyltryptamine, the main substance responsible for its visionary effect. The recreational abuse of these substances has increased in recent years, mainly because of their hallucinogenic effects.
In the present study, the concentrations of psychoactive alkaloids in three powder samples seized by the São Paulo State Police and nine ayahuasca beverages (aqueous extracts) prepared to religious rituals were analyzed by HPLC-DAD in an attempt to distinguish between illicit drugs and the religious beverage.
Nine ayahuasca beverage samples (prepared by a religious organization) and three powder samples (approximately 0.5 g each) seized by the São Paulo State Police were analyzed. For ayahuasca beverage samples, the materials were homogenized by vigorous mixing, centrifuged and diluted with methanol prior to HPLC-DAD analysis. For the seized powder samples, an amount (10 ± 1 mg) was weighed and transferred to a volumetric flask, followed by dilution with methanol. Chromatographic separation was performed with an Atlantis T3 column (150 x 3.0 mm, 3 µm) analytical column and gradient elution was done with (A) 10 mmol/L phosphoric acid in ultrapure water (pH adjusted to 3.0 with triethylamine) and (B) acetonitrile at a flow rate of 1 mL/min. Ten microliters were injected in HPLC-DAD system. Peak purity and UV spectra from reference materials were recorded and compared to those obtained in samples analysis, showing good agreement. Samples were quantified by standard addition. Seized powder samples were also analyzed using a validated gas chromatography-mass spectrometry drug screening method (standard procedure of our forensic chemistry laboratory).
The alkaloids detected in the ayahuasca beverage samples were N,N-dimethyltryptamine (402-2070 mg/L), harmaline (27.5-181 mg/L), harmine (295-2894 mg/L) and tetrahydroharmine (850-2053 mg/L), whereas of the two seized powder samples, one contained only N,N-dimethyltryptamine (82%, w/w) while the other contained both harmaline (16%, w/w) and harmine (12%, w/w).
We found a wide range of β-carbolines and alkaloid concentrations in ayahuasca bevarege samples obtained from a Brazilian religious organization. One of the powder samples seized by police presented high concentration of hallucinogenic DMT. The ritualistic use of ayahuasca involves oral intake and the probability of overdose risk is minimized by serotonergic stimulation of vagal pathways, leading to vomiting and diarrhea. In contrast, the recreational use of N,N-dimethyltryptamine involves consumption mainly by smoking or inhalation, both of which markedly increase its bioavailability and the potential for intoxications.

P208. ALCOHOL AND OPPORTUNISTIC DRUG FACILITATED SEXUAL ASSAULT

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Drug-facilitated crimes (DFCs) have increased substantially in recent times. These crimes are investigated when an individual reports having been robbed or assaulted while incapacitated due to the effects of a drug, usually a strong central nervous system (CNS) depressant. In the case of opportunistic DFCs the victim is already under the influence of a psychoactive substances consciously taken (primary CNS depressant, pCNS) when the offender administers in an underhand manner a second CNS depressant. The presence of a primary depressant dramatically extents the plethora of substances potentially able to induce a high degree of vulnerability of the victims. In particular many common and easy available medications can act as powerful depressant when the victim is already under the influence of alcohol.
The present study deal with the forensic toxicological investigation of opportunistic DFCs when alcohol is the primary CNS depressant. Pitfalls which could increase the risk of false negative results are emphasized.
Biological samples obtained from victims of suspected facilitated sexual assault (n = 4, female, 17 - 24 years) which admitted the voluntary use of alcohol were collected between 36 and 120 hours after the offence. Blood, urine and hair samples were analyzed through an extensive analytical protocol comprehensive of multiple analytical techniques. In particular a general unknown screening procedure based on high accuracy and resolution mass spectrometry were set up to detect a wide range of psychoactive molecules at nanogram level. Analysis were performed on a Orbitrap XL (Thermo) equipped with an ESI source operating in positive ion mode. Full-scan data were acquired at a resolution of 30000 (range m/z 100 - 600). Separation was performed on a Waters Atlantis T3 (150 x 2.1 mm, 1.8 µm) column. The adopted protocol proved to be suitable to identify anticonvulsants, antidepressants, antihistaminics, benzodiazepines, barbiturates, ketamine, GHB, z-drugs, in addition to the most common classes of drug of abuse.

An accurate anamnestic data collection indicated that the victims suffered of strong hangover, prolonged lack of memory, dizziness, fatigue, prostration on the days following the violence. Despite the similarity of the circumstances in which the assault took place and the side effects referred by the victims, only two cases the occulted administration of a CNS depressant was confirmed.
Toxicological analysis of opportunistic drug facilitated crimes is a difficult task particularly when alcohol is the primary depressant, indeed: 1) the assaulter has a plethora of psychoactive substances available to incapacitate the victim, many of that are over the counter medication; 2) as higher is the amount of alcohol ingested as lower is the dosage of secondary psychoactive substance needed to incapacitate the victim; 3) the strong hangover and the shame suffered by the victim often delayed the samples collection. In a such prohibitive scenario the risk of a false negative result can be significantly reduced, but not excluded, by an accurate choice of the analytical protocol and a strong collaboration between the toxicologist and all the other professional experts involved in the case.

P209. IDENTIFICATION OF THE MAIN METABOLITES OF MDMB-CHMICA IN HUMAN URINE USING UHPLC/MS/MS AND GC/MS TECHNIQUES

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MDMB-CHMICA (MMB-CHMINACA, methyl-(S)-2-(1-(cyclohexylmethyl)-1H-indole-3-carboxamido)-3,3-dimethylbutanoate) is recently one of the most widespread synthetic cannabinoid in Hungary, and became a controlled substance on January the 1st, 2015. We observed a large number of forensic cases when nothing or only trace amount of MDMB-CHMICA was detected in the urine although the blood sample proved to be positive for the unchanged drug. The biotransformation of this synthetic cannabinoid into more polar compounds was very likely. A presumed metabolite was first seen by HPLC-DAD.
The aim of this study was to find the possible metabolites of MDMB-CHMICA in human urine and to prove their chemical structure through mass spectra and derivatization techniques using Shimadzu UHPLC/MS/MS and GC/MS instrumentation.
Urine samples of suspected MDMB-CHMICA abusers were selected. We performed two different sample preparations. One was done with acidic hydrolysis, followed by solid phase extraction on BakerBond narc-1 cartridges. Aliquots of the extracts were analyzed using a Shimadzu UHPLC-DAD-MS/MS in scan mode to determine the molecular mass of the compound seen on HPLC-DAD. Then UHPLC/MS/MS was applied in product ion scan and multiple reaction monitoring (MRM) mode. The column was Kinetex C18 (100x3.0 mm, 2.6 µm), the eluents were acetonitrile with 0.1% formic acid and formic acid buffer pH 4. Other part of these extracts were derivatized with iodomethane and iodomethane-D3 and analysed by GC/MS (capillary column: Agilent VF-DA 12x0.20, linear velocity and splitless mode, acquisition mode: scan). The second way of sample preparation was performed in parallels by solid phase extraction on Agilent Bond Elut Plexa cartridges with and without enzymatic hydrolysis using Escherichia coli (IX-A) β-glucuronidase. These extracts were analysed using UHPLC/MS/MS in MRM mode.
The following ion fragments were found and set in MRM mode on the UHPLC/MS/MS corresponding to presumed metabolites: 371>144, 240, 97 (carboxylic acid metabolite); 401>256, 144, 95 (hydroxylated metabolites); 388>256, 144, 95 (hydroxylated carboxylic acid metabolites). The last two MRM transitions served two significant peaks with different retention times, which phenomenon refers to the existence of structural isomers. Hydrolysed and non-hydrolysed samples were compared and the degree of glucuronidation calculated. Applying GC/MS analysis derivatives of three main presumptive metabolites have been identified. One is the presumed carboxylic acid metabolite (methyl derivative: m/z 384 ($M^{\bullet+}$), 328, 240; trideuteromethyl derivative: m/z 387 ($M^{\bullet+}$), 331, 240). The other two are metabolites hydroxylated on the cyclohexyl ring (these are probably structural isomer; bis-methyl derivatives: m/z 414 ($M^{\bullet+}$), 358, 270; bis-trideuteromethyl derivatives: m/z 420 ($M^{\bullet+}$), 364, 273). Transesterification was intensive in both cases during derivatization. The use of N-Methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA) confirmed that the main metabolite is the carboxylic acid one (trimethylsilyl derivative: m/z 442 ($M^{\bullet+}$), 386, 240).
Five MDMB-CHMICA metabolites have been found by UHPLC/MS/MS. Three of them have been confirmed by GC/MS analysis. Methyl ester hydrolysis and hydroxylation on the cyclohexyl ring are the main pathways of the metabolism. The most abundant metabolite is the carboxylate derivative formed by ester demethylation of the parent compound. The main metabolites are significantly bound to glucuronic acid.

P210. FATAL HYPERNATREMIA DUE TO DRINKING A LARGE QUANTITY OF SHOYU (JAPANESE SOY SAUCE)

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Hypernatremia is defined as a plasma sodium (Na) level of greater than 145 mmol/L and represents a state of hyperosmolarity. Hypernatremia may be caused by water deficit or a primary Na gain. Hypernatremia due to water deficit accounts for the majority of cases of hypernatremia. Hypernatremia due to Na gain occurs infrequently.
We report the surprisingly small amount of salt that can cause severe hypernatremia.
A 55-year-old woman had a diagnosis of depression for 11 years. Her family doctor initiated antidepressive treatment. She was found lying on the floor with dark brown vomit around her mouth. A bottle of soy sauce was on a desk in her room. She had consumed approximately 700 ml of soy sauce (approximately 75 g of salt or 1300 mEq of sodium). Her caretaker took her to the hospital. She was deeply unconscious on arrival to the emergency department. She had a blood pressure of 100/40; respiratory rate, 40 breaths/min; heart rate, 128 beats/min; and temperature, 36.3 C. The estimated Glasgow coma scale score was 6. Her breathing was shallow and rapid. Pulmonary, cardiac,

and abdominal examinations were normal. The only available biological laboratory data were obtained at the moment of admission. These data showed the following values: serum sodium level of 187 mmol/L; chloride level of greater than 150 mmol/L; potassium level, 4.5 mmol/L; blood pH, 7.101; pO_2, 158.5 mmHg; pCO_2, 21.8 mmHg; standard bicarbonate level, 6.6 mmol/L of plasma; and base excess, 21.2 mmol/L of blood. The complete blood count and the rest of the biochemistry results were within normal range. The clinical examination of the lung fields revealed massive pulmonary edema that was confirmed by X-ray .
In the present case, the patient's death is attributable to massive pulmonary edema due to drinking a large quantity of soy sauce.

P211. EVALUATION OF PROCALCITONIN POSTMORTEM LEVELS IN SOME MODELS OF DEATH: AN EXPERIMENTAL STUDY

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Post-mortem determination of biochemical parameters especially for obscure cases, has been recognized as useful in establishing the diagnosis of the underlying cause of death, Procalcitonin (PCT) is the precursor of the calcitonin hormone, which is responsible for calcium homeostasis and it is a peptide that composed of 116 amino acids, which rises in a response to any proinflammatory stimulus.
The present study aims to estimate the postmortem levels of procalcitonin in the serum and some organs (kidney, liver, brain) in animal induced death models (traumatic, infection, drowning and freezing).
The study was performed on 60 male rabbits. Rabbits were divided into four different models induced death (15 rabbit each): the first was a trauma model, the second was a infection model, the third was a drowning model and the fourth was freezing model. At the end of the study, all rabbits were sacrificed; postmortem blood samples, kidneys, livers and brains were collected, and procalcitonin was measured using enzyme linked-immunosorbent assay.
Results showed significant increase in procalcitonin levels in all tested groups regarding different causes of deaths. Liver samples showed the highest levels in trauma, infection and freezing models, while in drowning model, brain levels of procalcitonin was the highest. Post Hoc multiple comparisons test for PCT levels showed significant differences between groups in most of liver, brain and kidney samples, while PCT serum blood samples were significant only between trauma and infection groups.
The results of the present study confirm that procalcitonin is allowing a differentiation between sepsis and non-sepsis related deaths. In addition, liver, kidney and brain procalcitonin could be an alternative to the serum procalcitonin for the diagnosis of postmortem sepsis.

P212. POST-MORTEM PRODUCTION OF PARACETAMOL VIA DEGRADATION OF ITS METABOLITES

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Paracetamol is one of the most widely used analgesic medications in the world. Taken in moderation, it is remarkably safe and free of side effects, however excessive amounts of paracetamol can cause severe liver damage and even death. For this reason, interpreting the concentration of paracetamol in the blood of a deceased person can be crucial in determining the cause of death. It has been observed that the concentration of paracetamol in post-mortem blood samples may increase in-vitro when not stored consistently at -20°C.
To determine the cause and extent of in-vitro paracetamol production in blood samples, with particular focus on the influence of paracetamol metabolites in this process.
Sample preparation involved precipitation of the blood samples with acetone and addition of salicylamide as internal standard. The supernatant was evaporated and reconstituted in 0.1% formic acid. All extractions were performed in triplicate. HPLC analysis was performed on an Agilent 1100 series HPLC equipped with diode array detector. Chromatographic separation was achieved on an Agilent Eclipse Plus C18 (50 x 4.6mm) column with a gradient system of 0.1% formic acid / methanol over 12 minutes. Recoveries for analytes ranged from 39-67% and calibration curves were linear up to 100mg/L (r2>0.995). Stability studies were conducted over a period of at least 30 days with samples taken approximately every second day, except in post-mortem blood where a limited supply of drug-free blood was available.
An investigation of three major metabolites of paracetamol (-glucuronide, -sulfate and -mercapturate) identified that paracetamol-glucuronide (P-Gluc) may be converted to paracetamol under certain conditions. P-Gluc was observed to be stable in aqueous solution at both high and low pH and at temperatures up to 40°C. A small amount of conversion to paracetamol was observed in ante-mortem blood samples stored at room temperature. However, in post-mortem blood samples, the conversion of P-Gluc to paracetamol was significant, even in samples preserved with sodium fluoride. In some unpreserved samples, complete degradation of P-Gluc was observed over a period of 5 days. Only those samples stored below -15°C showed no degradation of P-Gluc. Bacterial studies identified a range of species that can convert P-Gluc to paracetamol, with E. coli producing complete conversion in 24 hours.
Paracetamol-glucuronide has been shown to be converted to paracetamol in the presence of a range of bacterial species. The presence of sodium fluoride in the sample as preservative slowed this process, but storage below -15C was the most effective way of preventing conversion.

P213. **PLANT POISONING SHOULD BE CONSIDERED IN UNEXPLAINED SUSPICIOUS DEATHS. A CASE OF TAXUS POISONING.**

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Police investigation, internal body examination and a comprehensive systematic toxicological analysis on the tissue samples (blood, urine, stomach content, liver, kidney and bile) were unable to identify the cause of the sudden unexpected death of a 35-year-old woman.
The stomach content consisted of a vegetable-like, semi-solid mass (130 ml). On close inspection of the stomach content, partially digested foods, green-brown plant particles and two intact seeds were observed. The aim of the study was to investigate if plant poisoning could be involved using an ultra performance liquid chromatography tandem mass spectrometry method (UPLC-MS) for the multicomponent screening of plant toxins.
Aliquots of the stomach content were submitted to a liquid-liquid extraction with diethylether and ethyl acetate. The UPLC-MS system used was the Acquity separations module coupled to the Acquity TQD mass detector equipped with ES interface (Waters Milford, MA, USA). Chromatographic separation was achieved on an Acquity UPLC HSS C18 column at 50°C, using a gradient elution of 5 mM ammonium formiate pH 3 and 0.1 % formic acid in acetonitrile. Multiple reaction monitoring with positive ion detection and retention times were used for selective identification of plant-origin toxins.
The UPLC-MS method allowed the simultaneous identification of 20 plant toxins from regional and tropical origin. The method was successfully applied. Paclitaxel (Taxol A) was identified in the stomach content. Taxus poisoning was confirmed by a more extensive toxicological analysis. The toxic biomarker 3,5-dimethoxyphenol and taxoid cephalomannine (Taxol B) were qualitatively identified in the stomach content. These compounds were added to the screening method. Case history and analytical findings are presented.
Although uncommon, the ingestion of poisonous plants can be used as a method to commit suicide and should be considered in sudden unexpected deaths. In the presented case, no drugs and pharmaceuticals were detected by the routine toxicological analysis. A blood alcohol concentration of 0.18 g/L was measured. Toxic compounds of yew tree were identified in the stomach content. It was concluded that the woman's death was caused by yew tree (Taxus) poisoning. The manner of death was presumed to be suicide due to ingestion of the plant material.

P214. **SUSPICION OF TOXIC DEATH IF SUDDEN DEATH**

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Sudden death is an unexpected, non violent death that occurs suddenly, without evident cause, in subjects apparently in good health, without any medical or surgical history, often young.
Most often, it is a natural death, but the circumstances of its occurrence can makes it suspicious (e.g. when a body is found alone in a public place or in a house, or a playground ...) .
The medical doctor who is called to certify the death, mentions on the death certificate that the cause of death is undetermined, and this is an obstacle to the burial. Thereafter, a forensic autopsy will be ordered to determine the cause of death. Determination of cause of death in natural deaths, particularly when the death occurred suddenly, unexpectedly, or in the young, is an important part of forensic autopsy practice.
Sometimes, no cause appears on autopsy at the macroscopic or microscopic level, and there is necessity to complete investigations by forensic toxicological analyses of biological fluids and tissues to evidence or exclude a toxic origin of death (drug overdose, doping in young athletes ... etc.)
In the frame of post mortem toxicology, the biological samples that need to be taken at autopsy for a possible toxicological analysis will be reviewed, and the various possble causes of toxic death will be recapitulated.

P215. **A SUDDEN DEATH FROM TOXIC PSYCHOSIS**

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Methamphetamine is a psychoactive substance and is the second most widespread habit-forming drug in the Czech Republic. The abuse of methamphetamine carries many risks including the development of life-threatening toxic psychosis. This report is on the case of a young woman who was discovered dead in her flat. Her body showed signs of trauma and subsequent toxicological analysis revealed high concentrations of methamphetamine and other pharmaceutical drugs, which together could have contributed to the development of toxic psychosis and even death.
A 31-year-old woman was found dead in her flat. On examination of the body in situ by a medical examiner the suspicion was raised that the death was caused in connection with an attack by a second person. The body was found lying face down with multiple abrasions, sprains, contusions, lacerations and cuts. The furnishings of the flat had been destroyed and there were numerous bloodstains on the walls and furniture. The autopsy findings ruled out the possibility that death had been caused by the incurred injuries. The autopsy detected brain edema, hemorrhagic pulmonary edema and congestion of all organs. Samples of gastric contents, urine, blood and hair were taken for toxicological examination.
TLC and GC-MS methods were used for determination of drugs in biological specimens and GC-MS for quantification of drugs in blood. The isolation techniques used were diethylether extraction for qualitative analysis and SPE for quantitative analysis.
The presence of methamphetamine, ibuprofen, naproxen, citalopram and an antibiotic (clarithromycin) were proven

by the toxicological examination. The concentration of methamphetamine in the blood was determined to be 1.67 mg/l, amphetamine 0.78 mg/l, and citalopram 0.081 mg/l.
The cause of death of the 31-year-old woman was not her injuries as initially assumed, but a brain edema due to the effect of methamphetamine on her organism. This occurred simultaneously with ongoing toxic psychosis. The use of other substances (antibiotics and analgesics) which affect the pharmacokinetics of methamphetamine and can lead to overdose with these symptoms may also have been a contributing factor. In addition, the combination of methamphetamine with citalopram can cause serotonin syndrome, which could also have significantly contributed to the death of the victim.
The death of the young woman could have been caused by these significant factors: overdose with methamphetamine, toxic psychosis and the eventual development of serotonin syndrome due to the combination of methamphetamine with citalopram.

P216. DETERMINATION OF CARBOXYHEMOGLOBIN IN HEATED BLOOD: SAMPLE ANALYSIS IN THE LAC-MÉGANTIC DERAILMENT CASE

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On the night of July 6, 2013, an unattended freight train carrying petroleum crude oil derailed in the town of Lac-Mégantic, Québec, Canada. The incident resulted in the explosion of multiple tank cars and 47 people were fatally injured. Remains of victims were analyzed by the Laboratoire de sciences judiciaires et de médecine légale. Although determination of carboxyhemoglobin (HbCO) is routinely performed in cases of fire incidents, it has been reported that the results can be inaccurate if blood was exposed to high temperatures (>50°C). In the tragedy of Lac-Mégantic, victims were exposed to extreme conditions and were thus subject to a false elevation of carboxyhemoglobin levels.
Our goal is to illustrate the difficulty to measure %HbCO by spectrophotometry in intense fire victims.
Blood was available for 30 of the 47 victims and was sampled from the heart, the thoracic aorta or the iliac vein during autopsy. Samples were stored at 4°C in Starplex Leakbuster polypropylene containers without any preservative. Given the intense heat of the fire during the event, all blood samples were coagulated or carbonized. About 1 g of coagulated blood sample was diluted in 5 mL of 1 mg/mL sodium carbonate, vortexed and centrifuged 1 min at 2500 g. The supernatant was transferred to another test tube. Then, 20 mg of sodium dithionite and 500 µL of 1 N sodium hydroxide were then added to the supernatant, with vortexing after each addition. An aliquot of this solution was then transferred to a 1 cm path length disposable plastic absorbance cell. The absorbance spectrum from 500 nm to 650 nm was obtained using a double-beam spectrophotometer with sodium carbonate solution as the reference sample. The %HbCO was quantified by calculating the 560 nm/530 nm absorbance ratio.
The average of a duplicate %HbCO analysis was reported as the final result for each of the 30 cases. In 28 cases, the %HbCO was found to be above the lethal level (>50%), with a median of 93% (range 59 – 110%). The other two cases had a toxic HbCO level (23% and 49%). Since 5 cases were found to have a measured %HbCO above 100%, it is clear that at least some of these cases suffer from an interference that creates a positive bias. The interference of heated blood was visible on the spectrum when compared to a normal absorption spectrum. These observations cast a doubt on the results obtained for victims of the train derailment and of similar events.
Intense heat and thermo-coagulation of the blood cause interference in the determination of carboxyhemoglobin by spectrophotometry. Levels of HbCO can be falsely elevated in UV spectra with abnormal profiles and should not be used alone to determine cause of death.

P217. GLYPHOSATE INTOXICATION: A CASE REPORT

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Pesticides ingestion is one of the leading suicide methods. Among the pesticides the mortality rate due to suicidal poisoning with Glyphosate (GLYP) is usually low compared with the rates of organophosphate poisoning. In this case the deceased was an 36 year old man who was found dead in a field. There was a container of liquid around him. The bottle of liquid contanined Glyphosate Isopropylamine.A forensic autopsy was performed after his death. Blood sample was taken for toxiclogical analysis.
The aim of the present study was the determination of Glyphosate in postmortem blood by using a gas chromatography-mass spectrometry (GC-MS) method.
We used the Gas Chromatography Mass Spectrophometry method for determination of glyphosate in blood. GLYP (1 mg/ml) prepared in 10% methanol. 7 point calibration curves constructed at concentrations covering (1, 5, 10, 20, 50, 75, 100 µg/ml.). Blood sample was extracted by employing a solid-phase extraction(SPE) technique utilizing a Sep-Pak Plus PS-2 sorbent, obtained from Waters Corporation, along with a styrene-divinylbenzene sorbent. After eluation, the blood sample was reconstituted in 50 ul each of MTBSTFA with 1% TBDMCS and acetonitrile, and the mixture was vortexed (15 min.) 1 µg aliquat of derivatized sample was injected into the GC-MS system. GC-MS analyses were performed using an Agilent 5973. A 30 m x 0.25 mm HP-5MS , 5% diphenyl 95% dimethylsiloxane capillary column with a film thickness of 0.25 µm obtained from Agilent was used.
The calibration curve of GLYP was linear between the concentration ranges from 1 to 100 µg/ml. Glyphosate level of the blood was found as 19,95 µg/ml.
Although GLYP analysis is not typically included in the systematic toxicological analysis, its wide use in society makes the analysis of the GLYP in different matrices necesseray.

P218. ACUTE INTOXICATION OF METHOMYL AND AGRICULTURAL SPREADING AGENT

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A male aged 35 patient hospitalized for car accident was suspected of carbamate pesticide ingestion. After hospitalization he died and conclusively, he might be complicatedly intoxicated with methomyl, methanol, ethanol and agricultural spreading agents. The additives and solvents of pesticides, and agricultural spreaders have toxic effects itself or different toxic profile from active compounds and were more toxic than active compounds in some pesticides. The liquid products of methomyl contain methanol as a solvent. The metabolites of methanol may cause metabolic acidosis, blindness, and death after a characteristic latent period of 6~30 hours. Methomyl is highly toxic carbamate pesticide which can cause muscarinic, nicotinic and central nervous system side effects. Polyoxyethylene alkyl aryl ethers (POEAAEs) is used in agricultural spreading agents including that have non-specific toxicity. We concluded he was intoxicated with methomyl, methanol, ethanol and spreaders, and methanol would be the main cause of toxicity base on their kinetic and toxic profiles.
The aim of this case study was to find out major cause of death in complicated intoxication.
The quantitations of methanol and ethanol were achieved with an Agilent GC-FID with a headspace method. For the analysis of POEAAEs, methomyl and methomyl-oxime, we used LC/MS/MS system. Biological specimens were extracted directly with ethyl acetate in basic condition. The LC system was coupled to a 3200 QTrap mass spectrometer using electrospray ionization in positive mode and multiple reaction monitoring mode. Liquid-chromatographic analysis was performed in gradient mode on ADME column (shiseido capcell core, 2.7 μm, 100 x 2.1 mm). The mobile phase was consisted of A: 0.5% aqueous acetic acid and B: 0.5% acetic acid acetonitrile. The flow rate was 0.2 mL/min.
The methanol, ethanol, methomyl, methomyl-oxime and POEAAEs concentrations were 360 mg/L, 0.154%, 9.1 mg/L, 1.72 mg/L and 1.87 mg/L in the peripheral blood during treatment. The methanol, ethanol, methomyl, methomyl-oxime and POEAAEs concentrations were 505 mg/L, 0.031%, 0.26 mg/L, 2.58 mg/L and 0.70 mg/L in the peripheral blood in autopsy samples, respectively. The methomyl and methomyl-oxime concentrations were 32.53 and 3.76 mg/L, and POEAAEs was not detected in aqueous humor of autopsy samples. The methomyl-oxime and POEAAEs concentrations were 3.76 and 0.50 mg/L in the heart blood of autopsy samples while methomyl was not detected. The POEAAEs concentration was estimated as 360 mg/L, but methomyl and methomyl-oxime were not detected in a part of stomach contents.
The methanol and methomyl-oxime were increased, whereas ethanol and methomyl were decreased in the peripheral blood in autopsy samples than those from the treatment. The methomyl was not detected in heart blood and stomach contents. This means that at first, he took alcohols and methomyl but he lived for some period. After then, he might drink Gramoxone inteon supplemental agent® contain POEAAEs. Early period ethanol might initially inhibit the metabolism of methanol. He was intoxicated with methanol, methomyl and agricultural spreaders, but his death would be mainly caused by metabolic acidosis due to methanol.

P219. THE USE OF PERICARDIAL FLUID OR SKELETAL MUSCLE IN POSTMORTEM TOXICOLOGY – WHAT DOES THE LITERATURE SAY?

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In postmortem toxicology peripheral blood is considered the gold standard when concentrations of drugs of relevance to the case are to be determined. However many other biological materials will come into use when peripheral blood is lacking, or when more than one medium is needed in order to better interpret the findings in difficult cases. Pericardial fluid and skeletal muscle are sometimes utilized in our laboratory when blood samples are lacking due to blood loss or putrefaction. We do, however, only report that compounds are detected, not the concentrations when such materials are used. Skeletal muscle is found in abundance, is easily accessible and probably less prone to postmortem redistribution. Skeletal muscle is present even in most cases of severe putrefaction. Pericardial fluid has mainly been used in order to determine cotinine levels in cases of sudden infant deaths where blood is difficult to collect. It is fairly easily sampled and analyzed, and the question is whether it more often could be utilized for drug analysis. However, the question remains how well drug concentrations in such media correlate to concentrations in peripheral blood.
To perform a systematic review of the scientific literature on the use of pericardial fluid or skeletal muscle in determining postmortem drug concentrations.
Literature searches were conducted in MEDLINE and EMBASE in 2013. The search strategy consisted of words related to postmortem drug concentrations and drug analysis regarding drugs of abuse and other potentially toxic drugs normally analysed for in postmortem cases at the Norwegian Institute of Public Health's division of Forensic Sciences.
Only 10 studies comparing concentrations in pericardial fluid to femoral blood, and 28 studies comparing skeletal muscle to femoral blood were identified, suggesting that these media are poorly investigated. Several of these studies were case-reports with few cases studied. Also few studies exist from recent years. Several of the studies found a relatively good correlation between pericardial fluid as well as skeletal muscle compared to blood with respect to drugs studied, such as morphine, cocaine and ethanol.
Few studies have investigated the issue; however it seems that postmortem drug concentrations both in pericardial fluid and skeletal muscle could correlate well with concentrations in blood. Such matrixes might thus be collected as alternative specimens in more postmortem toxicology cases. However larger studies are needed to enhance our experience with such matrixes.

Poster abstracts

P220. **INTERACTION BETWEEN KETAMINE AND ETHYL ALCOHOL MAY CAUSE DEATH???**

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An Estonian model was found murdered in Rawa Island off Malaysia's east Coast. The Freelance model was initially thought to have drowned when her body was discovered on beach along island. The police later classified the case as murder case after discovering suspicious wounds on victim's body. Even though the cause of death determined by pathologist was multiple blunt force trauma, toxicology analysis were still required to assist the investigation. For this purpose, autopsy specimens consists of blood collected from common iliac vein, bile, urine, vitreous fluid and stomach content specimens were sent to Department of Chemistry Malaysia.
To determine the presence of drugs, poison, Lysergic acid diethylamide (LSD) and ethyl alcohol level in specimens submitted.
An acidic and basic extraction were carried out using liquid-liquid extraction techniques on specimens submitted to the laboratory and further identified using Gas Chromatography Mass Selective Detector (GCMSD). A quantitation of Ethyl Alcohol and Ketamine were performed using Gas Chromatography Flame Ion Detector (GCFID) and Gas Chromatography Nitrogen Phosphorus Detector (GCNPD) respectively followed by analysis of Lysergic Acid Diethylamide (LSD) using Liquid Chromatography Mass Spectrometry Mass Spectrometry (LCMSMS).
The results revealed that Ketamine and its metabolite, Norketamine were detected in blood, urine, stomach content and bile specimens submitted. Ethyl Alcohol was also found in blood (90 miligram/100 mililiter), vitreous fluid (91 miligram/100 mililiter) and urine (101 miligram/100 mililiter) specimens. As for Ketamine level, 5.24 microgram Ketamine was quantified in blood specimen. Lysergic Acid Diethylamide (LSD) was not detected in stomach content, blood and urine specimens submitted.
The implication of this study suggest that the Ketamine lethality risk is probably increasing in combination of alcohol consumption. One might wonder that the real cause of death maybe due to particular pharmacokinetic interaction and or to synergistic effect of Ketamine and Ethyl Alcohol. However further study need to be done to evaluate the risks associated when combining Ketamine with ethyl Alcohol and other unanticipated consequences associated with poly drugs use.

P221. **OPIUM POPPY RELATED DEATHS IN TASMANIA FROM 2011-2014**

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Tasmania grows over 50% of the world's licit poppy crop for pharmaceutical uses. A variety of visually indistinguishable poppy breeds are grown within Tasmania, each with differing concentrations of target opioid alkaloids. There are many opium alkaloids within the opium poppy including morphine, codeine and thebaine, many of which are present in low concentrations. Theft and subsequent consumption of opium poppies has been reported as well as death due to opiate overdose. We have identified 3 deaths involving opium poppy consumption, and present our findings.
The aim of the study was to investigate and quantitate the major opiate compounds present in the post-mortem biological specimens (e.g. blood, vitreous humour, urine) from three individuals who had died following suspected consumption of illicitly obtained opium poppies.
Post-mortem biological samples including blood, vitreous humour and urine were selected for analysis, though not all matrices were available for each subject. Three extraction methods were employed including two different basic liquid/liquid extraction methods and a mixed-mode solid phase extraction. Some extracts were derivatised (PFP, TMS, butylation) for analysis. Extracts were analysed by GC/MS (SIM and TIC modes) and UPLC/MS (separation by PFP column with gradient elution, positive ion-spray ionisation in MRM mode on QTrap mass spectrometer)
Case Study 1 – 51 year old male. Post-mortem blood sample contained morphine (0.4 mg/L), codeine (< 0.05 mg/L) and thebaine, with neopine indicated by library search. Case Study 2 – 17 year old male consumed boiled poppy juice. Post-mortem blood contained morphine (0.2 mg/L), codeine (0.05 mg/L) and thebaine. Poppy juice contained codeine, morphine and thebaine. Case Study 3 – 26 year old male (Danish tourist) consumed poppy tea from poppy heads. Post-mortem blood contained morphine (0.4 mg/L), codeine (< 0.06 mg/L) and thebaine. Vitreous humour and urine contained morphine, codeine and thebaine. Initial targeted screening detected morphine, codeine and thebaine in the blood of all three post-mortem samples. 6-MAM was not detected within these samples. Thebaine, an opioid alkaloid used to commercially manufacture oxycodone, is significantly more toxic than morphine. Identification of thebaine in these cases is a significant finding, especially with the absence of 6-MAM, a major heroin metabolite, as it indicates opium poppy consumption, rather than heroin. The visually indistinguishable nature of poppy varieties, further enhances the risk of mixed drug toxicity. Identification of other opium poppy alkaloids continues.
Three cases were reported in which morphine, codeine and thebaine were detected following ingestion of products concocted from illicitly obtained commercial poppy varieties. The combination of opioids detected, in particular thebaine, as well as the absence of heroin metabolites, provides evidence of opioid poppy consumption rather than administration of pharmaceutical opiate or opioid formulations, or heroin use. Further investigation is required to adequately detect other opioid alkaloid constituents, or associated metabolites, to distinguish between opium poppy consumption and to further exclude heroin or pharmaceutical opioid product consumption.

P222. **EVALUATION OF POST MORTEM AMINO ACID CONCENTRATIONS IN VITREOUS HUMOR WITH KNOWN POST-MORTEM INTERVALS**

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Estimation of the post mortem interval (PMI) is often so called the Holy Grail of forensic researches.
The aim of this study was to investigate the correlation between free amino acid concentrations and known post-mortem intervals in 135 vitreous humor samples.
135 samples of vitreous humor (from the right eye) obtained from autopsies at the Intitute of Forensic Medicine of the University of Bonn with known post-mortem intervals (n=10 PMI=0-9 days, n=9 PMI 10 days, n=8 PMI=11 days, n=1-4 PMI=12-21days) were analysed using HILIC LC-MS/MS. Sample preparation of 10µl vetreous humor was performed using protein precipitation with acetonitrile. The following amino acids can be detected with the descrided method and are related to their labelled IS: alanin, asparagine, aspartic acid, glutamine, glutamic acid, histidine, isoleucine, leucine, ornithine, phenylalanine, tryptophane, tyrosine and valine. Calibration range was 5 µg/ml - 1000 µg/ml. Determination of leucin and isoleucine was possible with mass spectrometric and chromatographic separation.
The concentration of each amino acid increased with rising post-mortem interval up to 21 days. The best correlation coeffients of linear regressions were R = 0,87 for aspartic acid and R = 0,83 for tryptophane. Medians of each PMI showed even better correlation, however, standard deviation was high. In comparison to the linear regression the the best correlation coeffients of polynomial regressions of the medians were R = 0,98 for aspartic acid and R = 0,96 for L-leucine. In case of aspartic acid the median concentration increases from 8 µg/ml (PMI=0) up to 297 µg/ml (PMI=21). This corresponds to an increase of approximately 10 µg/ml per day.
This post mortem study showed that there is a direct relationship between the formation of free amino acids in vitreous fluid and the degradation time after death. A still existing proteolytic activity after death in vitreous humor seems to be the cause of a degradation of endogenous proteins into free amino acids.

P223. **AMIODARONE FOR RESUSCITATION; EXPECTED POST-MORTEM CONCENTRATIONS**

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Amiodarone is an anti-anginal drug prescribed for the prevention of ventricular arrhythmias and may also be administered in emergency situations as per current UK Resuscitation Council Guidelines (2010). Amiodarone has a prolonged plasma half-life of 14-107 days and is metabolised to its active metabolite desethylamiodarone. Current maintenance dose therapeutic reference ranges for amiodarone and desethylamiodarone are 0.9-12mg/L and 0.3-2.7mg/L respectively. In our post-mortem toxicology workload we have observed cases in which the history clearly details amiodarone use for resuscitation rather than chronic condition management. Amiodarone and desethylamiodarone have been quantified in these cases.
To undertake a review of cases from 2013-14 in which amiodarone and desethylamidoarone were quantified, and determine their expected concentration ranges in cases where amiodarone was used for resuscitation purposes compared to the proposed maintenance dose reference ranges.
All cases were subjected to an initial unknown drug screen using LC-MS/MS, including the detection of amiodarone and desethylamiodarone. In the 45 positive cases, quantitation of amiodarone and its metabolite were performed by LC-MS/MS following protein precipitation with methanol using deuterated analogues as internal standards.
There were 45 cases in which amiodarone and/or desethylamiodarone were detected and subsequently quantified. Of the 45 cases, 24 (53%) were categorised as amiodarone employed for resuscitation (using details given in the histories provided). These cases included incidents such as post cardiac arrest, individuals involved in road traffic accidents or detailed attendance of paramedics/attempts at resuscitation. The amiodarone concentrations in these cases ranged from <0.1 to 3.3 mg/L (median 1.2 mg/L). Desethylamiodarone in all but one of these cases was below the reportable limit of 0.1 mg/L. The one case with a quantifiable desethylamiodarone had a very low concentration of 0.2 mg/L. This pattern of results is suggestive of therapeutic intervention immediately prior to death rather than long-term drug usage. The concentrations of amiodarone reported following resuscitation intervention fall mainly within the bottom quartile of the reported therapeutic range.
Due to the incomplete medication histories provided, only five cases could be categorised as likely to be chronic/long-term amiodarone usage. Unfortunately this is not sufficient to draw any conclusions. However it is noteworthy to mention that the active metabolite desethylamiodarone was elevated above the quoted therapeutic range (0.3-2.7mg/L) in two of these cases, something which was not observed in any of the cases where amiodarone was used solely for resuscitation.
In some instances it is easy to decipher whether amiodarone has been used for resuscitation. In these cases we are currently reporting the therapeutic reference ranges for amiodarone and its active metabolite. The use of these ranges is not justified given that one off use of amiodarone invariably results in an amiodarone concentration between <0.1 and 3.3 µmol/L. Desethylamiodarone concentrations are usually below the lower limit of detection.
It can be difficult to distinguish between those cases where amiodarone has been used long-term versus resuscitation. In these cases desethylamiodarone may be a useful indicator in conjunction with the history provided.

Poster abstracts

P224. POSTMORTEM REDISTRIBUTION OF DONEPEZIL IN RATS

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At our institute, high concentrations of donepezil hydrochloride (DPZ) are detected in the blood of human cadavers. However, postmortem drug concentrations in blood may not always reflect the antemortem drug concentration in blood because of the redistribution of drugs after death.
In this study, the concentrations of DPZ in postmortem cardiac blood and tissues (brain, heart muscle, lung, liver, and thigh muscle) of rats were measured. We also examined the postmortem redistribution and changes in postmortem tissue distribution of the drug. In addition, we investigated the relation between pH and the accumulation/release of DPZ in/from the tissue.
We have chosen the rat as an animal model. The rats received 5 mg/kg DPZ through intraperitoneal injection once a day for 10 days. They were sacrificed at 3 h after the last injection, and the cadavers were left at room temperature for 0, 1, 3, 6, 12, and 24 h, at which times blood and tissue samples were taken. Drug concentrations in tissues and blood were analyzed with time-of-flight mass spectrometry. The pH of cardiac blood was measured.
The tissue-to-cardiac blood concentration ratio at 0 h was higher in the lung (left lung: 46.05 ± 30.42, right lung: 48.10 ± 34.04 [mean ± SD]) and in the liver (left liver: 54.55 ± 25.85, right liver: 59.98 ± 39.58). DPZ was also well distributed in the brain, in which the tissue-to-blood concentration ratios were >1. The DPZ concentration in cardiac blood at 0 h was 51.31 ± 34.03 ng/mL; at 1 h, 164.46 ± 88.23 ng/mL; and at 24 h, 719 ± 208.73 ng/mL. The DPZ concentration in cardiac blood was increased by approximately 3 times at 1 h and 14 times at 24 h compared with that at 0 h. The pulmonary concentration decreased quickly from 3 h after death and then did not vary. A decrease of about 50% was observed between 0 and 24 h in the left and right lungs. For the other tissues (liver, heart, brain, and thigh muscle), the concentrations did not change ($p > 0.05$). The pH in cardiac blood decreased with time: from 7.3 ± 0.1 at 0 h to 6.1 ± 0.1 at 24 h.
On the basis of the tissue-to-blood concentration ratios at 0 h, DPZ is distributed preferentially in the lungs, liver, and brain. Our results show that the cardiac blood and lung tissue concentrations of DPZ change during the postmortem period. It is inferred that DPZ in the lung is redistributed into cardiac blood through pulmonary venous blood after death with the decrease of pH, contributing to the increase in its concentration in cardiac blood. As the concentration did not change significantly in the brain, liver, heart, and thigh muscle, these tissues were considered to be less prone to postmortem redistribution. The application of the data from a rat study to a human population should be carried out with caution. However, although there may be some differences about the time and degree, postmortem redistribution of DPZ was considered to have occurred in humans.

P225. LEVAMISOLE AND COCAINE SYNERGISM: A WIDESPREAD ADULTERANT ENHANCES COCAINE'S TOXICITY

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Levamisole has been identified as a cocaine adulterant in the United States since 2002. By 2009, the United States Drug Enforcement Administration (DEA) estimated that 70% of seized cocaine contained levamisole. Although there is a variation in the percentage of levamisole in cocaine samples between European countries, the trend is an increase of these percentages, this has become a large public health concern, measurement of levamisole in human samples of cocaine users has become increasingly important. To our best knowledge, only five deaths are reported (one twice) as a result of complications secondary to levamisole-tainted cocaine and none of these cases reports the postmortem levamisole concentration in all biological samples. Moreover, the cases reported here concern three men died after levamisole-adulterated cocaine intake, no other drugs were consumed by this subjects. Most of other reports in literature regard polydrug addicts, in which it is more difficult to discern the real cause of death. Levamisole-induced complications in chronic cocaine abusers are widely identified in literature, but what is it known about the acute toxicity of the peak blood levamisole?
The Authors present for the first time in literature the postmortem levamisole concentrations in all biological fluids and tissues in three exclusive cocaine users, showing how, along with cocaine, this adulterant may play a significant role in triggering the events leading to the exitus. This article aims to be a supplementary alert to aware the risk that may occur using levamisole-adulterated cocaine and an incentive to publication of toxicity reports and new research involving the combination of levamisole and cocaine.
In all cases were evaluate autopsy, histopathological and toxicological findings, which allowed us to exclude other pathologies as well as different causes of death. Mainly toxicological analysis, performed on the viscera and body fluids, give the most important data.
Positive qualitative and quantitative results were obtained for cocaine, benzoylecgonine and levamisole in blood, urine, gall, gastric content, brain, lung, liver, kidney and hair. No aminorex was detected in all the biological specimens analyzed.
The postmortem levamisole concentrations led us possible to affirm that the peak blood levamisole detected in these cases can be attributed to its metabolism, which is slower than cocaine. Therefore, in cases of fatal binges of cocaine cut with levamisole, cannot be excluded that the high blood concentrations of the adulterant played an important role in the mechanism of death, reaching toxic doses. The lack of this toxicological data could not allow to properly analyze the role of levamisole in pathogenetic mechanism of toxicity. Whereas cocaine increases dopamine at the synaptic level through its blockade of reuptake, levamisole as a nicotinic agonist may work on glu-

tamate containing neurons at the level of the cell body of the dopaminergic neuron, indirectly stimulating it through the release of excitatory glutamate. Thus, interactions of levamisole at central neuronal nicotinic acetylcholine receptors may explain the linkage with cocaine that can result in a greater chance of toxicity on heart and CNS.

P226. UNUSUAL SUICIDES IN PHYSICIANS: TWO CASES INVOLVING FENTANYL AND PHENOBARBITAL.

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Fentanyl is basic in pain treatment and anesthesiology. Phenobarbital is a GABA-modulator used as hypnotic, sedative, anesthetic and anticonvulsant. Both of them can produce physical dependence, abuse and overdose. Literature unusually reports self-poisoning by Fentanyl, and, rarely, in the last three decades by Phenobarbital, especially in suicide.
Two cases of unusual suicides acted by physicians, one by Fentanyl and one by Phenobarbital, are presented.
Case 1: A 64-year old retired anesthesiologist was found dead in his countryside house. Two bottles (100 mL each), one empty and the second one half filled with a clear liquid, were found near the body hanging from the chandelier, while a needle was stuck into his left ankle. Case 2: A 65-year old retired cardiologist was found dead at home. A needle was stuck into his left median cubital vein, connected with an empty syringe (20 mL). Eight empty boxes of Gardenale (Phenobarbital) (20 x100 mg tablets) and two empty boxes of 6 vials (1 mL) of Pantopon (a mixture of all natural alkaloids of opium), produced in 1962, were found near the body. In both cases autopsy findings showed no evidence of natural disease or trauma. Biological samples collected at autopsy and paraphernalia seized at death scene were tested by systematic toxicological analysis (STA) using GC-HS (FID), GC-MS and LC-MS/MS for alcohol, most common drugs and pharmaceuticals.
Case 1: Fentanyl was revealed in the bottles and in all biological specimens. Its concentration was: blood 30.6 µg/L, urine 87.9 µg/L, bile 70.2 µg/L, liver 0.094 µg/g; brain: 0.064 µg/g. Norfentanyl was present only in the blood and liver suggesting a rapid death. Comparing these data with those reported by the literature, the death was listed as a suicide by Fentanyl. Case 2: Toxicological analyses performed on biological samples were positive for Phenobarbital, Morphine and Codeine. Phenobarbital was present in blood (527.5 µg/mL), urine (106.3 µg/mL) and liver (208.0 µg/g) at concentration above therapeutic levels. In addition Morphine was detected in blood (0.14 µg/mL), urine (2.2 µg/mL), bile (70.7 µg/mL) and liver (53.6 µg/g) associated with Codeine in blood (0.11 µg/mL), urine (1.2 µg/mL), bile (0.75 µg/mL) and liver (0.22 µg/g). The analysis of residues found in the syringe were positive for Phenobarbital, Morphine, Codeine, Papaverine, Noscapine and Hydrocodone. Based on the toxicological results the cause of death was listed as acute intoxication by Phenobarbital. Furthermore, positive results for opiates (Morphine and Codeine), in bile more than in blood, suggested a chronic consumption of opiates.
A review of the literature on acute poisoning using Fentanyl and Phenobarbital was applied for the toxicological evaluation of these suicides, performed with an unusual manner.

P227. QUANTIFICATION OF 25C-NBOME IN A FATAL POISONING CASE - IDENTIFICATION BASED ON A DEMETHYLATED AND GLUCURONIDATED METABOLITE OF 25C-NBOME

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Recently, a new class of hallucinogenic drugs termed NBOMe has appeared on the drug market and has gained prominence. They have no recognized therapeutic use, and adverse negative health effects have been reported. NBOMe drugs are analogs of the 2C series of psychedelic phenethylamine drugs that contain an N-o-methoxybenzyl ("NBOMe") substituent, which significantly affects their pharmacological activities. The three most frequently reported drugs in this series are 25I-NBOMe, 25B-NBOMe and 25C-NBOMe. Prior to 2010, essentially no history of the human use of drugs of the NBOMe class had been reported on the internet. Since then, reports of intoxication and deaths associated with the intake of these drugs have increased. 25I-NBOMe seems to be the most widely abused drug of the NBOMe derivatives, and signs and symptoms of 25I-NBOMe intoxication have been described in several scientific papers. However, there are few scientific reports of intoxication with the other NBOMe-derivatives. Recently, drugs of the NBOMe class have entered the Scandinavian drug scene, and in the present work, we describe the first death related to the intake of 25C-NBOMe in Denmark. Since October 2013, this drug has been controlled in Denmark. To the best of our knowledge, the present work reports the first quantification of 25C-NBOMe in biological specimens obtained from a fatal intoxication case.
Quantification of 25C-NBOMe.
Ultra-performance liquid chromatography with high-resolution time-of-flight mass spectrometry (UPLC-HR-TOFMS)and UPLC with tandem mass spectrometry (UPLC-MS/MS).
In the present case, a young male was hospitalized after the recreational use of 25C-NBOMe. He died at the hospital at approximately 12 hours after ingestion. Medico-legal autopsy was performed on the deceased and biological samples were submitted for toxicological examination. 25C-NBOMe and a demethylated and glucuronidated metabolite of 25C-NBOMe were identified in the urine and blood samples using UPLC-HR-TOFMS. Subsequently, 25C-NBOMe was quantified in the peripheral whole blood (0.60 µg/kg), urine (2.93 µg/kg), vitreous humor (0.33 µg/kg), liver (0.82 µg/kg) and gastric content (0.32 µg total) samples by using a validated UPLC-MS/MS-method. The clinical course and autopsy findings were consistent with serotonin toxicity. Based on these findings and the toxicological findings the cause of death was determined to be a fatal overdose of 25C-NBOMe in combination with amphetamine.

The high potencies of 25C-NBOMe and other similar drugs challenge even highly sensitive methods, as HR-TOFMS, because the signals from these drugs are very low and easily lost in the background noise of the sample. The number of NBOMe related intoxication or fatal cases may well be underestimated due to the lack of proper routine analytical methods used. In the present case, the detection of 25C-NBOMe was made possible by information provided by the police. In the future, the detection of drugs of the NBOMe class in our laboratory is improved by screening for their demethylated glucuronidated metabolites, using UPLC-HR-TOFMS.

P228. DOMINO EFFECT: SINGULAR CASE OF SIX FATAL HYDROGEN SULFIDE POISONINGS IN QUICK SUCCESSION. EVALUATION OF SULFIDES QUANTIFICATION METHOD.

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Hydrogen sulfide is one of the major toxic gases in forensic practices (Maebashi et al., 2011, Forensic Sci Int); it is a colorless gas and has a strong odor of rotten eggs. Aside from being a by-product of many industrial processes, this gas is naturally produced during the putrefaction of organic substances. Hydrogen sulfide (H_2S) is absorbed by the upper respiratory tract mucosa and it causes histotoxic hypoxemia and respiratory depression by exerting an inhibitory effect on cytochrome oxidase. We report 6 autopsy cases of fatal hydrogen sulfide poisonings due to the inhalation of hydrogen sulfide gas because of an occupational accident. In fact, six men died during an unblocking of a wastewater cistern. The first worker died shortly after clearing the occlusion, the other five died one by one in an attempt to help colleagues.
The goal of this study is to evaluate the role of toxicological data in the possibility to distinguish between the H_2S concentration in blood secondary to lethal poisoning and the endogenous H2S produced during the putrefaction. For this purpose, we compare the post mortem H_2S concentrations of the 6 men died at work, with the post-mortem concentrations of endogenous H_2S in blood samples of 12 subjects died for different causes. At autopsy, femoral blood samples were collected at different post mortem interval (PMI), we divided the 12 subjects according to the PMI in three groups (first group: 24-36 hours; second group: 37-72 hours; third group: 73-120 hours).
0.2 ml of blood sample was added to a mixture of 0.5 ml of 20 mM PFBBr solution in toluene, 2.0 ml of internal standard solution (10 mM of TBB in ethyl acetate) and 0.8 ml of 5 mM TDMBA solution in oxygen-free water saturated with sodium tetraborate. The preparation was vortexed for 1 min, and 0.1 g of potassium dihydrogenphosphate was added to the mixture. The preparation was again vortexed for 10 s and centrifuged at 2500 rpm for 10 min. An aliquot of the organic phase was then injected into the GC/MS apparatus.
The results of this study showed femoral blood H_2S concentrations (n = 6 poisoning) ranged from 8.7 to 28.6 mg/L and femoral blood endogenous H_2S concentrations (n = 12 died for other casues) ranged from 2.2 to 32.7 mg/L. A significant relationships between PMI and H_2S concentration was observed. In the 12 blood samples analysed, in fact, sulphide blood concentrations were already detectable at 24 hours after death.
The performed analysis led us to affirm it is not possible, only on the basis of toxicological data, to distinguish between the H_2S concentration in blood secondary to sulphide lethal poisoning and the H_2S produced during the putrefaction. This presentation will impact the forensic community for the importance of circumstantial evidences along with the histopathological findings (greenish-blue color of the hypostasis and of the internal organs, of the brain in particular, related to post mortem formation of sulfhemoglobin) used to identify H_2S lethal poisoning about the pathological pathway leading to the related death.

P229. A 20 YEAR RETROSPECTIVE STUDY OF CYANIDE INTOXICATION CASES

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Cyanide is a powerful chemical asphyxiant. It acts mostly through fixation to cytochrome C oxidase, the mitochondrial enzyme responsible for the last step in cell respiration, and to hemoglobin, the blood oxygen transporter. Cyanide intoxication is most often encountered in suicide or murder cases. These cases are rare, most likely because access to cyanide is not easy nowadays. The majority of intoxications occur via ingestion of a cyanide salt (KCN or NaCN), although inhalation of HCN was also reported in a suicide case.
The aim of this project was to compile toxicology results and autopsy findings for cyanide intoxication cases in the province of Quebec.
The information contained in the pathology and toxicology files of cyanide intoxication cases that occurred between 1995 and 2015 was retrieved and compiled. All gastric contents during that time frame were screened for the presence of cyanide using a pump with a Draeger-Tube filled with $HgCl_2$ and methyl red. The indicator turned red in the presence of HCN. A colorimetric screening method, using a Conway cell and o-dinitrobenzene, was used for the screening of other matrices. Prior to 2011, cyanide in biological matrices was quantitated spectrophotometrically. Briefly, a diluted aliquot of the sample was acidified with tartaric acid to generate volatile HCN gas which was caught in a $NiCl_2$ trap. A $NiCN_4$ complex was formed in solution and analyzed by UV-Vis spectrophotometry. Post-2011, the quantification method was changed to a published headspace GC-MS method using $_{13}C_{15}N$ as an internal standard (ISO 17025 compliant).
A total of eight (8) cyanide intoxication cases occurred in the province of Québec between 1995 and 2015. All of them were Caucasian males between 32 and 77 years old. Most were found dead at home with a suicide note and a container of a cyanide salt or solution. Only one case was a suspected murder. Cyanide concentrations ranged from 2.41 to 127 µg/mL (median 19.5 µg/mL) in cardiac blood and from 0.3 to 7.5 µg/mL (median 4.05 µg/mL) in femoral

blood. The mass of cyanide found in the gastric content ranged from 1 to 500 mg (median 36 mg). Other xenobiotics detected in biological matrices included ethanol, acetaminoiphen, codeine, methotrimeprazine, morphine, venlafaxine, clonazepam and THC. The most common autopsy findings were heavy and congested lungs with edema, cherry-red blood and lividities and hyperaemia of the gastric mucosa. A strong scent of bitter almond was also noted by the restricted number of pathologists and assistants with the ability to smell cyanide.
The toxicological and pathological findings compiled from these eight cases can be added to the corpus of knowledge for cyanide intoxications. This information can help assess new suicide or murder cases by cyanide.

P230. PREPARING POSTMORTEM BLOOD BY "QUECHERS" EXTRACTION METHODS FOR LC-MS/MS ANALYSIS OF DRUGS AND TOXIC COMPOUNDS

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Analysis of drugs and toxic compounds in postmortem blood is complicated by the presence of hemolyzed blood products and a wide variety of analytes of interest (often at low concentrations) with basic/acidic, hydrophilic/hydrophobic characteristics. Having noted successful applications of the "QuEChERS" (quick, easy, cheap, effective, rugged, and safe) extraction methods to the analysis of pesticide residues in food and agricultural products, we have conducted this study to develop an optimal QuEChERS method for effective extraction of drugs and toxic compounds from postmortem blood samples for LC-MS/MS analysis.
To develop a modified QuEChERS method for analysis of drugs and toxic compounds in postmortem blood samples. The modified QuEChERS approach involved a 2-step process, i.e., extraction/partitioning and dispersive-solid phase extraction (d-SPE). In step 1, 1-mL aliquots of blood sample were extracted by six different QuEChERS methods, each partitioning into three layers by centrifugation. In step 2, each of the six resulting top extract layers was processed with three different d-SPE sorbents, followed by centrifugation. Supernatants derived from these processes (a total of 18 combinations) were analyzed by LC-MS/MS to evaluate the recoveries of the analytes of interest. A mixture of 31 forensically relevant drugs (including opiates, amphetamines, cocaine, benzodiazepines) and 23 case samples were included in this study; results were compared against those derived from the Toxi-tubes® A liquid-liquid extraction (LLE) method, that has been established and routinely used in our laboratory.
The modified QuEChERS method included the use of inorganic salts helpful to blood coagulation and isolation of the organic extract phase. Combination of 1-mL Na_2CO_3/$NaHCO_3$ buffer, 0.8-g anhydrous $MgSO_4$ (as dehydrating agent), 0.2-g NaCl (as salting-out agent), 2-mL acetonitrile (as organic solvent), and the d-SPE cleanup sorbent (containing 25-mg PSA, 25-mg C18EC and 150-mg $MgSO_4$) provided optimal sample pretreatment products. Recoveries of the 31 analytes (each at 0.5 µg/mL) ranged from 56 to 78%, except morphine (40%) and benzoylecgonine (33%). Application of this modified QuEChERS and the LLE methods to the analysis of 23 casework postmortem blood specimens generated a combined total of 168 positive results of 84 compounds; 85.1% and 82.7% of these positives were reported by the modified QuEChERS and the LLE methods, respectively. For drugs that were detected by both methods, their quantitative data were in good agreement.
A modified QuEChERS method, operated under alkaline condition, has been successfully developed to pretreat postmortem blood for LC-MS/MS analysis of drugs and toxic compounds. New abuse drugs, such as 4-chloroamphetamine, 5-MeO-MiPT, and PMMA, can also be detected with this approach. With low cost and easy to use, this approach can potentially become the preferred cleanup method for the analysis of drugs in postmortem blood sample.

P231. SIMULTANEOUS DETERMINATION AND QUANTITATION OF FENTANYL, NORFENTANYL, ALFENTANIL, AND SUFENTANIL IN POSTMORTEM BLOOD AND URINE BY LC-MS/MS

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Fentanyl (F), norfentanyl (NF), alfentanil (AF), and sufentanil (SF) are short-acting and highly potent µ-opioid agonists that are widely used for anesthetic and analgesic purposes. Therapeutic levels of these fentanyl-like compounds are as low as 1 ng/mL in plasma, therefore it's important to develop a sensitive method for detection and quantitation.
Since these synthetic compounds are often present in postmortem specimens from accidental, suicidal, and homicidal poisoning cases, we have developed a simple and sensitive LC-MS/MS method for their analysis in postmortem samples to assist medical examiners in determining the cause of death.
2 mL of 1.5 M Na_2CO_3/$NaHCO_3$ (pH = 9.5) buffer solution was added to 1 mL of blood or urine samples containing the analytes's deuterated analogs, F-d5, NF-d5, AF-d3, and SF-d5 — quantitatively added to serve as internal standards. Liquid-liquid extraction was performed with 3 mL dichloromethane/1,2-dichloroethane/n-heptane/ethyl acetate (1:1:1:1, v/v) mixture. Chromatographic separation was achieved using an Agilent Zorbax SB-Aq (100 mm x 2.1 mm i.d., 1.8-µm particle) analytical column at 50 oC. The mobile phase consisted of 0.1% formic acid (v/v) in water (A) and methanol (B) at a flow rate of 0.32 mL/min. Mass spectrometric analysis was performed under electrospray ionization in positive-ion multiple reaction monitoring (MRM) mode. The precursor and two transition ions (m/z) adopted for F, NF, AF, and SF were 337, 188/105; 233, 150/84; 417, 268/197; and 387, 238/111, respectively. Corresponding precursor and transition ions (m/z) for F-d5, NF-d5, AF-d3, and SF-d5 were 342, 188/105; 238, 155/84;

420, 271/200; and 392, 238/111, respectively.
Drug-free blood and urine samples, fortified with 2–40 ng/mL of the four analytes of interest, used for method validation yielded the following results: (a) average extraction recoveries ranges: 67.04–98.64% for blood, 58.93–98.90% for urine; (b) intraday and interday precision ranges (percent CV): 0.37–3.31% and 0.77–8.55%; (c) intraday and interday accuracy ranges: 88.93–104.6% and 92.37–106.3%; and (d) calibration linearity (r2), detection limit (LOD), and quantitation limit (LOQ): >0.999, 0.01–0.1 ng/mL, and 0.01–0.1 ng/mL, respectively. LOD was defined as the lowest concentration at which ion ratio pairs monitored for a particular analyte fell within ±20% of that observed in the standard; while LOQ was defined as the lowest concentration at which LOD requirements were met and the observed concentration also fell within ±20% of the expected value. Observed ion suppression was about 30% for F and NF; 35% for AF; and 45% for SF. This phenomenon was closely monitored — and found adequately compensated for — when the analytes' deuterated analogs were used as the internal standards for quantification.
Among 3740 toxicological cases during the 2014– Feb. 2015 periods in our institute, 18 blood specimens were found to contain at least one of these four compounds with the following means and concentration ranges (ng/mL): F (6.51, 0.18–29.10); NF (1.80, 0.09–5.79).
The validated protocols are easy and quick to carry out, and have been successfully utilized to the analysis of these fentanyl-like compounds in postmortem samples.

P232. TRENDS IN POLY-DRUG AND COCAINE USE IN DRUG-RELATED DEATHS IN CAMPANIA DURING 2008 - 2014

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The drug-related death is a phenomenon that, in the various geographical areas, can be characterized by significant differences in the incidence and prevalence of substances involved.
The trends in the use of cocaine and of more than one drug among the drug related deaths in the Campania Region, since 2008 to 2014, were studied.
The study regards n. 320 drug related deaths (94% males and 6% females) observed at the Forensic Toxicology Unit of the Second University of Napoli (SUN) – Italy, in the period 2008 - 2014. For each case, autopsy findings and all drugs detected were recorded. Moreover age, race/ethnicity, gender, place and circumstance of death were collected in order to study the territorial dynamics about demand and the supply of drugs. A systematic toxicological analysis (STA) for ethanol, illicit drug and pharmaceuticals has been applied on the biological specimens routinely collected at autopsy and all positive results were confirmed by a quantitative analysis performed by GC-HS (FID), GC/MS or LC-MS/MS.
The toxicological results show that simultaneous use of multiple illicit drugs was responsible of the 56.9% of deaths. In these cases the cocaine was the most detected substance (75,2%) while as drug used alone is responsible only of the 4.7% of all deaths. Particularly in the last two years, the finding of methadone is increased from the 30.2% (2008-2012) to 50% of cases, among the deaths related to more than one substance. Conversely the prevalence of Cannabis that regarded 22.3% of deaths, during 2008-2012, is decreased to 14.3%. Furthermore the results about the different incidence, in the considered period, of opiates, ethanol, new drugs and amphetamines were investigated in both cases of poly-drug use and single-drug use. In the distribution by age and gender, males aged over to 40 years and women older than 35 years old, are prevalent. The territorial distribution of the deaths in the different surrounding areas and in the districts of the Naples city demonstrates that the 74% of deaths occurred in a place different from the usual residence.
Our results show that among the drug related deaths, in Campania Region, trends in overall mortality reflect a prevalence of the poly-drug use with an increasing involvement of Cocaine and Methadone. Furthermore, the integration of toxicological findings with the other circumstantial data allowed to obtain a territorial map of the risks related to the supply and to the consumption of illicit drug.

P233. SUICIDE AS MANNER OF DEATH AMONG USERS OF SYNTHETIC CATHINONES

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Synthetic cathinones are a group of novel psychoactive substances that have gained widespread publicity over the last decade. They are structurally related to cathinone, the principal active compound of khat. In Finland, the most prevalent of the abused synthetic cathinones have been MDPV, methylone and α-PVP, all of which are currently banned. One of the synthetic cathinones, namely bupropion, is in medicinal use for the treatment of depression and as a smoking cessation aid, which makes its user profile quite different from that of the other synthetic cathinones. Previous studies have suggested high prevalence of suicides among deceased positive for synthetic cathinones. Additionally, reports suggesting increased incidence of suicide and suicidal thoughts among some patients treated with bupropion have been published.
By looking at the post-mortem cases positive for synthetic cathinones in Finland during a five-year period (2009-2013) we sought to find evidence of a potentially elevated incidence of these substances in suicides.
In the study period, comprehensive post-mortem toxicology was performed in about 6000 death cases yearly representing about 13% of all fatalities in Finland. In all relevant cases, screening for both traditional and new psychoactive substances in urine was performed by ultra-high performance liquid chromatography coupled with high-resolution time-of-flight mass spectrometry, followed by quantification in blood and urine by appropriate mass spectrometric target methods. In this register-based study, all cases positive for synthetic cathinones were

examined in terms of toxicological data, background information, and the circumstances of the death.
There were 128 cases positive for synthetic cathinones during 2009-2013. Six different synthetic cathinones were detected, bupropione, MDPV, alpha-PVP, mephedrone, methylone, and pyrovalerone. In nine cases, more than one of the synthetic cathinones was present. Among the 128 cases, the manner of death was accident in 38.7% of cases and suicide in 35.8% of cases. Bupropion dominated the suicide statistics with over 55% of the cases being suicides. The majority of all suicides (79.2%) were fatal poisonings. In more than half of these, the forensic pathologist had implicated the particular synthetic cathinone in the cause of death.
The proportion of suicides in cases positive for synthetic cathinones was very high when compared to the proportion of suicides among users of illegal drugs in general. In most cases the deceased had used several different psychoactive substances, which complicates the interpretation of these findings. It is, however, safe to conclude that users of synthetic cathinones seem to be more vulnerable to suicide than users of the more traditional drugs. There are significant differences in the action mechanisms between the studied substances just like in their corresponding phenethylamine analogues which probably explain some of the differences observed. However, our results support earlier findings suggesting that synthetic cathinones are highly prevalent in suicides and raise concerns of the adverse effects of bupropion in medicinal use.

P234. RETROSPECTIVE STUDY OF BLOOD ALCOHOL CONCENTRATIONS IN VICTIMS OF SUICIDE BY HANGING IN SOUTH KOREA

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Suicide is the fourth leading cause of death in Korea. Hanging is the most common suicide method in South Korea. Although a variety of psycho-social factors affect the rate of suicide, heavy alcohol drinking is associated with a loss of judgement, increasing impulsive and destructive behavior, which might lead to the triggering action of suicide.
The aim of this study was to analyze epidemiologic profiles of the tendency for predisposed individuals to commit suicide.
A cross-sectional retrospective study was conducted by collection of data from autopsy reports for victims of suicide by hanging in Korea, in the year of 2013 (N = 234 cases).
The age range was 13-93 years. Mean ages (standard deviation, SD) of the victims was 44.9 ± 15.9 years with a clear predominance of males 64% (mean age 45.9 ± 15.9 years) compared with 36% females (mean age 43.2 ± 15.6 years). Blood alcohol concentration (BAC) was detected (>0.10 g/L) in femoral blood to avoid contamination of cardiac blood from stomach. A positive BAC was found in 48.3%, with 49.3% and 46.4% in male and females, respectively. The mean BAC for all positive victims was 1.36 ± 1.02 g/L with a similar values for females and males (1.34±1.01 g/L for males; 1.40±1.06 g/L for females).
The collection of these data serves the need for nationwide strategies to enhance suicide prevention.

P235. AN ACCIDENTAL INTOXICATION BY PHOSPHINE. THREE DEATHS IN THE SAME FAMILY

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Aluminium phosphide is a cheap solid fumigant and a highly toxic pesticide which is commonly used for grain preservation. When aluminium phosphide comes into contact with humidity, it releases large quantities of hydrogen phosphine (PH_3), a very toxic gas. Exposure generally occurs by way of accidental or suicidal ingestion. In this case three members of the same family died by inhalation from the manipulation of lids with aluminium phosphide dust residues.
A crime investigation has been made to determine the cause of the three deaths. Early symptoms were non-specific and abnormal physical findings included ataxia, intention tremor, and diplopia. All these three patients had severe toxic effects such as shock, cardiac arrhythmias, pulmonary oedema and renal failure. Very high blood lactic acid level (>20mmol/L) without evidence of hypoperfusion, suggested a cellular hypoxia as the mechanism of death. The deaths occurred between 8 and 12 hours after suspected inhalation, despite all efforts to avoid. After the autopsy, death could not be attributed to any organic or violent cause and toxicological analysis of biological samples and histopathologycal study of tissues was carried out. Two large sacks with hundreds of lids (997), seemingly cleaned, were found in the house.
Post-mortem specimens were collected at the autopsy. After the addition of water, the samples were immediately sealed in airtight vials. For the determination of phosphine a SPM extraction and gas chromatographic analysis/mass spectrometry (GC/MS) were carrying out. Other blood, urine and vomiting samples were checked for presence of ethanol, pesticides, cyanide, ethylenglycol, carbon monoxide and drugs of abuse using routine methods including immunochemical tests and solid-phase extraction procedures, with subsequent analysis by gas chromatography/mass spectrometry (GC/MS) which ruled out any other cause of death. Dust residues on lids were analyzed for phosphine in accordance with the procedure set out before. Aluminium and phosphorus was analyzed by ICP-AES in biological fluids. Addition standard method was applied.
Phosphine gas was absent in the blood, urine and vomiting samples at limit of detection of the method applied. Another toxicological analyses carried out in the different victim's samples were negative too. Blood and urine

aluminum and phosphorus concentrations were as nearly normal in all of the victims compared to the usual published values.Histopathological examination revealed the presence of megamitochondrias in liver. Phosphine was detected in the dust residues on the lids found in the house. Although phosphine could not be detected in biological samples, the cause of death was attributed to accidental phosphine intoxication. Due to the following facts: presence of hundreds of lids with aluminium phosphide residues at the crime scene, clinical sings and symptoms and histopathological examination results compatible with highly hypoxic compound and negative results obtained in the comprehensive toxicological examination performed. This unfortunate case draws attention to the need for strictly controlled disposal of highly toxic products especially in a situation of economic crisis, as it is suspected that phosphine was released during the process of tampering lids prior to the possible fraudulent re-selling.

P236. METHADONE IN HAIR: CONTAMINATION OR POISONING. REPORT OF 2 CASES

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Contamination of hair (head hair or pubic hair) from body fluids (sweat or putrefactive fluids) could be an issue in interpreting drug concentrations in post-mortem hair.
The aim is to report the methadone and EDDP concentrations that could be measured in body fluids and hair segments of an exhumed body and in pubic hair after acute intoxication with methadone.
Body fluids were submitted for a general unknown screen by GC-FID, GC-MS and LC-DAD. Hair (20 mg) were decontaminated twice by dichloromethane and finely cut; methadone and EDDP deuterated i.s. were added. Hair specimens were incubated overnight in pH 7.6 Soerensen buffer at 56°C. Extraction was performed with dichloromethane/ether. Concentrated extracts were analyzed by LC-MS/MS in SRM mode. Detection limits were respectively 20 and 10 pg/mg in hair for methadone and EDDP.
Case#1: a 21-year-old female was found dead on the bank of a river. Despite a lot of contusions, no visible cause of death could be found at autopsy. Alcohol was detected in blood (0.52 g/L), vitreous (0.28 g/L) and urine (1.77 g/L); methadone and EDDP were also detected in blood and in the urine. An acute intoxication by methadone and alcohol was suspected. Three years later, a suspect was arrested, and the body was exhumed. Victim's hair (50 cm) was submitted to analysis in order to determine a possible long-term abuse of methadone. Case#2: one year later, a 31-year-old female was found dismembered and partially burned at an edge of a wood. Autopsy revealed a huge pulmonary edema and that the victim was dead before to be burned. Cardiac blood and bile were submitted for analysis. Alcohol was detected in blood (2.32 g/L), together with methadone and EDDP. Poisoning by methadone was suspected, and 3-mm pubic hair was analyzed.

	Case #1				Case #2	
	Head hair (exhumation) (pg/mg)			Blood (µg/mg)	Pubic hair (pg/mg)	Blood (µg/ml)
	0-6 cm	6-18 cm	18-50 cm		3 mm	
methadone	102	114	153	0,71	2520	0,53
EDDP	53	36	48	0,03	38	0,02

In view of the results we hypothesized that the victim in Case#1 was abusing occasionally but on long term low doses of methadone, and that the victim in Case#2 was on methadone treatment or abuse. However the circumstances surrounding the deaths and extensive police investigations showed no signs of methadone addiction or previous misuse. Thus the possibility of an external contamination of the hair should absolutely be considered. It is clearly possible that head hair sampled 3 years after death could have been contaminated by putrefactive body fluids, and that pubic hair (3mm length) could have been polluted by intensive sweat after acute intoxication with methadone combined with alcohol.
Our data are in accordance with those previously reported in the literature for methadone. The low and homogenous concentrations of methadone and EDDP detected in segmental hair analysis could be indicative of external contamination by putrefied body fluids. Also, a high concentration could be due to contamination by sweat with an acute exposure close to the time of sampling.

P237. QUANTIFICATION OF BUPRENORPHINE IN POST-MORTEM BLOOD BY TWO-DIMENSIONAL GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Buprenorphine is a semi-synthetic opioid. It is chemically similar to morphine but it's potency is up to 40 times greater. Buprenorphine has been used successfully for the management of moderate to chronic pain and for opioid dependency treatment. Some analysts classify less than 5 ng/mL in plasma as therapeutic while others suggest 2 - 20 ng/mL. There has been a substantial rise in illicit availability of buprenorphine on the black market. Since the introduction of the higher dose buprenorphine, 14 fatalities have been reported in the UK. It has In post-mortem toxicology the experimental method should therefore be sensitive enough to detect buprenorphine at therapeutic concentrations.

The aim of this study was to validate a method for the quantification of buprenorphine in post-mortem blood using solid-phase extraction and two-dimensional gas chromatography-mass spectrometry (2D GC-MS) and to demonstrate method applicability to authentic post-mortem blood samples.
Sample preparation consisted of a two-step protein precipitation process using acetonitrile. Buprenorphine was purified using mixed mode (C8/cation exchange) solid-phase extraction cartridges. Endogenous water-soluble compounds and lipids were removed from the cartridges before the samples were eluted, concentrated and derivatised using N-methyl-N-trimethylsilyltrifluoroacetamide. The samples were analysed using 2D GC-MS.
The assay was linear from 1.0 - 50.0 ng/mL (R2 > 0.99). Intra-day and inter-day imprecisions (%RSD) were less than 5% and the average recovery was 60%. The limit of detection was 0.5 ng/mL and limit of quantification was 1.0 ng/mL. 2D GC-MS improved the limit of detection for buprenorphine by 20-fold compared to analysis on a conventional GC-MS. Concentrations of buprenorphine in 14 post-mortem blood samples ranged from 1.8 - 43.0 ng/mL.
2D GC-MS demonstrated increased sensitivity for buprenorphine analysis compared to conventional GC-MS. We report a simple, efficient and cost-effective solid-phase extraction 2D GC-MS method for the quantification of buprenorphine in post-mortem blood. 2D GC-MS is a complementary method to both conventional GC-MS and LC-MS/MS.

P238. DECOMPOSITION KINETICS OF DDVP IN BURIED CADAVERS

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A number of Dichlorvos (dichlorovinyl dimethyl phosphate, DDVP) poisoning cases are currently taking place in China. The decomposition kinetics of DDVP in stored dog specimens and in buried dog cadavers were studied. This study was supported by the National Key Technology R & D Program of China (2007BAK26B05 and 2012BAK02B02).
To observe the decomposition kinetics of DDVP in stored blood and buried dog cadavers.
The blood of six poisoned death dogs, thirty nine buried poisoned death dogs were prepared. After the storage or burial for 0 h~20d or 365d, DDVP in stored dog blood, or buried dog body fluids and tissues were detected qualitatively by GC/MS and quantitatively by GC/NPD with a linear range of 0.1-20μg/g(μg/mL) and a LOQ of 0.015μg/g(μg/mL).
After storage for 20d, no DDVP could detected in all dog blood stored at 20°C, 4°C, -20°C and 20 °C (1%NaF). DDVP concentrations detected in dog blood stored at -20°C was were higher than at 20°C and 4°C (T-test, $p<0.05$). After the burial for 104 d, no DDVP was detected in all collected tissues of buried dogs except for in stomach, for 365d, no DDVP for all. The buried way and dosage showed affections on the detection of DDVP in buried dog cadavers.
The decomposition kinetics of DDVP in stored dog blood and buried dog cadavers can be useful in assisting to interpret postmortem DDVP and DDVP concentrations for medico-legal cases potentially involving DDVP poisoning.

P239. THE RELEVANCE OF THE DETERMINATION AND QUANTIFICATION OF ETHANOL IN DIFFERENT MATRICES: A CASE REPORT

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Although the determination and quantification of ethanol is preferably carried out on blood samples, the determination of this parameter in other biological matrices can prove to be quite important. This may be true, for example, in cases where the blood sample available is insufficient or is in a state deemed unsuitable (e.g. putrefaction or contamination by other biological fluids such as gastric contents). Among the biological samples which can be considered as an alternative to blood in the determination of ethanol, the vitreous humor appears as the most interesting. We report the case of a 10 year old child autopsy that showed a positive value of ethanol in the blood. The autopsy was held two days after death, showing signs of putrefaction (extensive green abdominal stain). The clinical information highlighted the existence of a "lung infection", apart from Larsen syndrome and cerebral palsy. By these reasons, the search of ethanol was made in the vitreous humor, obtaining a negative result.
The objective of the presentation of this case report was to demonstrate the relevance of ethanol research in the vitreous humor, in situations where, because of the existence of putrefactive phenomena, the determination of this substance in the blood may not be adequate.
The analysis was performed using an Agilent 6890N gas chromatograph equipped with a flame ionization detector and coupled to an Agilent G1888 headspace injector of fixed volume (1 ml) (HS-GC/FID). Prior to gas-chromatography analysis, all specimens, including the calibrators, were diluted 1:10. With this purpose, 100 μL vitreous humor or blood were diluted with 1 mL aqueous solution of n-propanol (100 mg/L), used as internal standard. Lower limits of detection (LLOD) and quantification (LLOQ) of ethanol were, respectively, 0.02 g/L and 0.08 g/L, and the method was linear in the concentration range of 0.1- 5 g/L (R^2>0.99). Inter and intraday precision were in conformity with the criteria normally accepted in bioanalytical method validation.
Quantification of blood ethanol in this case revealed a value of 0.38 g/L. The research of this substance in vitreous humor showed a negative result.
Failure to detect the presence of alcohol in the vitreous humor, associated with the low blood alcohol level observed and the body showing signs of putrefaction in the context of an infectious process, favors the hypothesis of an endogenous production of alcohol. The authors enhance the importance of the analysis of different biological matrices, including peripheral blood, urine and vitreous humor, among others that may be necessary for a better forensic assessment of such cases.

Poster abstracts

P240. FATAL POISONING OF A CHILD BY AN OUT-OF-DATE DIAZINON AND ITS DEGRADATION PRODUCTS

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This case presents a fatal poisoning of a 17-month old child in Castellón (Spain). He was admitted to a hospital and was diagnosed with cardiorespiratory arrest. He was treated but he did not survive. The suspected toxic was a severely degraded ant-killing product (Diazinon) which had been stored at home. Diazinon is an organophosphorus insecticide classified by WHO as "moderately hazardous" Class II. It is known that a poor storage of emulsifiable Diazinon concentrate can produce a degradation of Diazinon to pyrophosphates with high toxicity (OS-TEPP, SS-TEPP), when it is contaminated with a small amount of water. In the last decades of the 20th century there were several important poisoning cases of livestock (lamb) with degradation products of Diazinon.
The goal of this study is to present the distribution of Diazinon and its different degradation products among gastric content, cardiac blood, peripheral blood, kidney, liver, lung, bile, pericardial fluid and vitreous humour.
An qualitative analytical investigation was performed on the different necropsy samples and fluids collected by the coroner and sent to our laboratory to be studied. The original container was also sent with some residue of the product, labelled "Diazinon 60% p/v, Líquido Emulsionable". The analysis was performed by GCMS after extraction of all biological samples received. Furthermore, the residues found in the container and clothes (trousers, sweater and T-shirt) were also analysed. The limit of detection (LOD) of diazinon was estimated to be 100 µg/L, based on 3 times of the signal/noise ratio. The residue found in the container and on the clothes was positively identified as Diazinon with its degradation products and impurities. The substances found in the container and clothes in the order of elution, were:Diethyl methyl phosphate (1); Phosphoric acid, triethyl ester (2); Trietylphosphorothioate (3); O,O'-Diethyl S-methyl thiophosphate (4); Methyl 2-isopropylpyrimidine-5-carboxylate (5); Phosphoricacid,triethylester (6); Tryethylphosphorothiolate (7); 2-isopropyl-4-methoxypyrimidine (8); 5-isopropyl-2-ethylthio-3-methylpiperidina (9); Tetraetyl pyrophosphate, TEPP (10); Hydroxy diazinon (11); Monothiono tetraethylpirophosphate, OS-TEPP (12); 5-acetyl-2-methylthiopyrimidine (13); Dithionotetraethylpyrophosphate, SS-TEPP, Sulfotep (14). The distribution of the different substances among the biological samples was the next. Gastric Content: (1), (2), (5), (6), (7), (8), (9), (12), (13), (14); Cardiac blood: (1), (2), (5), (6), (7), (8), (9), (12); Peripheral blood: (7), (8), (9), (12); Kidney: (5), (7), (8), (9), (12), (14); Lung: (1), (2), (5), (6), (7), (8), (9); Liver: (1), (2), (5), (6), (7), (8), (9), (12), (14); Bile: (1), (2), (5), (6), (7), (8), (9), (12); Pericardial fluid: nothing detected; Vitreous humour: nothing detected.
The products with higher distribution among the biological samples were triethylphosphorothiolate (7), methyl-6-hydroxypyrimidine (8) and 5 isopropyl-4-methoxypyrimidine (9). OS-TEPP was found in all the biological samples except lung, pericardial fluid and vitreous humour but SS-TEPP was only found in gastric content, kidney and liver. Any of investigated products was detected in pericardial fluid and vitreous humour. It was impossible to establish the limits of detection of each of the 14 products studied at the moment of the incident because the lack of standard.

P241. FEASIBILITY IN THE ESTIMATION OF THE MICROBIALLY PRODUCED ETHANOL IN POSTMORTEM CASES BY MATHEMATICAL MODELS

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The approach of modeling the microbial ethanol production enables an approximation to the quantification of the microbial ethanol production in cases where other low molecular weight alcohols are produced simultaneously with ethanol. The basis of this idea was that although the postmortem or putrefactive conditions could be extremely different and variable among different cases, the patterns of other alcohols would follow, more or less, in qualitative and quantitative terms, the ethanol production, since the biochemical pathways of their microbial production are interactive. Therefore the patterns of ethanol and alcohols produced under different, real conditions could be approximated by the models, and this is why ethanol estimation could be feasible within an acceptable standard error.
To test the feasibility of the application of the constructed models in real cases (postmortem blood and urine), that were positive for ethanol and other alcohols (measured by HS-GC-FID) during the original ethanol analysis.
Retrospective review of our chromatograms archives from postmortem cases and selection of those that had positive ethanol concentrations and co-detection of higher alcohols and 1-butanol during the original ethanol analysis. Chromatograms of 77 blood and 12 urine analyses were selected. The developed previously models [Boumba et al., 2011; 2013] were applied to calculate the microbial produced ethanol concentrations in each case. Standard errors produced for each case after comparing the original ethanol concentrations measured for each case and the calculated concentrations after applying each model were used to evaluate the applicability of the models.
At least one model for each case provided estimation of the microbial ethanol concentration within an acceptable standard error of 40% compared to the measured ethanol concentration in the majority of the tested cases (95%). The models could be applied both in cases of blood and urine analyses. Better scores in predicting the microbial produced ethanol by the models were achieved in cases having original ethanol concentration lower than 0.7 g/L. In general, lack of adequate evidence in respect to the origin of ethanol for the cases did not allow a definite conclusion, although putrefaction and extensive trauma of the body contributed to ethanol production.
Each model represents a "tool" which could be used as an alternate choice to estimate the microbial produced ethanol in real cases. In every given case, the effectiveness of each model in achieving the goal is different (due to the different postmortem conditions, resulting in different alcohol patterns); thus, one "tool" could be more effective and accurate than the others for each case. Ideally, a model should exist for every postmortem case (predisposing that it was constructed under the same conditions) which is impossible, since the conditions could not be defined accurately.

P242. A CASE OF FATAL CAFÉ CORONARY: AETIOLOGIES OF CHOCKING

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Café coronary is a sudden and unexpected accidental occlusion of the airways by food during a meal, leading or not to death. The case of a schizophrenic patient found dead by chocking is reported. Mr X, 62 years, back home after a short hospitalization, suddenly collapsed. The death was recorded by the Emergency services.
The aim of the study was to determine the origin of chocking.
The autopsy took place 48 hours later. Biological samples (peripheral blood and urine) were analysed: ethanol by CPG-FID, drugs screenings by HPLC-DAD and GC-MS. Quantification was performed by HPLC-DAD in peripheral blood.
During autopsy, a sizeable objet (orange quarter 8 x 4 cm) was found at the beginning of the larynx, fitting closely the pharynx and the beginning of the larynx. The origin of the death was chocking by complete obstruction of the airways. The toxicological analyses showed: absence of ethanol, 5 psychotropic drugs and an active metabolite: amisulpride (3037 µg/L), cyamemazine (382 µg/L), zuclopenthixol (85 µg/L), amitriptyline (181 µg/L) and nortriptyline (206 µg/L). Tropatepine was identified but not quantified. All concentrations were therapeutic except for amisulpride. Amisulpride concentrations until 1100 ng/ml were reported for patients with therapeutic high daily doses (1200 mg/day = daily dose in our case). A high concentration could also be due to post-mortem redistribution, as described for antipsychotic drugs in peripheral blood. Café coronary risk factors were identified: age (>50), cerebral disease or psychiatric disorders, polyphagia, altered dentition, xerostomia, psychotropic drugs. Neuroleptics, even at therapeutic doses, are able to cause dysphagia by several mechanisms: bradykinesia of extrapyramidal syndrome (EPS), tardive dyskinesia (oro-pharyngo-oesophageal), acute laryngeal or oesophageal dystonia. Anticholinergic drugs are not always effective on EPS dysphagia. But, a few café coronary syndrome cases are reported in psychotic patients without treatment. In our case, the drugs identified in blood were those prescribed. No previous dysphagia was notified in the hospital records. This patient accumulated several risk factors: age, psychiatric disorder, antipsychotic drugs (3 neuroleptics). His pathology, with an at-risk behavior, can alone cause café coronary. Nevertheless, the potential role of neuroleptics in chocking cannot be discarded.
The autopsy findings, the clinical history and the toxicological analyses results allowed us to diagnose a fatal café coronary syndrome. The origin can be an at-risk behavior, the influence of antipsychotic drugs being difficult to assess. To prevent swallowing disorders or chocking in psychotic patients, a complete investigation must be performed to search risk factors and organic and/or iatrogenic aetiologies such as clinical examination, upper gastrointestinal endoscopy, removal of the neuroleptic drug.

P243. A LIQUID CHROMATOGRAPHY-ELECTROSPRAY TANDEM MASS SPECTROMETRY METHOD FOR THE DETERMINATION OF PROPAFENONE IN FORENSIC WHOLE BLOOD SAMPLES: A CASE REPORT

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In forensic toxicology, antiarrhythmic drugs are of considerable interest because these substances may induce adverse side effects or worsen arrhythmias that may cause life-threatening situations or death. Propafenone is an antiarrhythmic substance that exhibits β-adrenergic and calcium channel-blocking activities. The authors present a fatal case involving a 62 year-old male, found dead in bed by his son, with vomitus around his body. No signs of violence were found at the death scene and only the presence of empty medication blister packs was noted. He had a previous history of alcohol detoxification and was under antiarrhythmic and diabetes mellitus treatment.
The aim of this work was to develop a validated liquid chromatography-tandem mass spectrometry assay for the quantitation of propafenone in whole blood.
Chromatographic analysis was preceded by an optimized solid-phase extraction procedure using Oasis® HLB (3 cc, 60 mg) extraction columns. The extracted analytes were separated by UPLC (Waters) with a reversed-phase Acquity UPLC® HSS T3 (2.1x100 mm id, 1.8µm) column in a gradient mode (0.1% formic acid and acetonitrile) at a 0.5 mL/min flow rate and a chromatographic run-time of 8 min. The Waters TQD triple quadrupole LC/MS system was operated under the multiple-reaction monitoring mode (MRM) using the electrospray ionization technique in positive mode. Lower limits of detection (LLOD) and quantification (LLOQ) of propafenone were, respectively, 0.03 ng/mL and 0.11 ng/mL, (respectively) and the method was linear in the concentration range of 10-1000 ng/mL (R2>0.999). Both within- and between-day coefficients of variation were lower than 10%. No carryover or interferences were observed. Toxicological results showed high blood concentrations of propafenone (1378 ng/mL) and tiapride (5760 ng/mL) and therapeutic concentrations of oxazepam and nordiazepam. Blood sample analysis also revealed the presence of ethanol at a concentration of 0.22 g/L. Neither illicit drugs nor pesticides were present in the analysed samples.
The LC-MS/MS method showed to be appropriate for screening, identification and quantification of propafenone in blood after intake of toxic or therapeutic doses. Toxicological results led the pathologist to rule that death was due to an intoxication caused by the simultaneous ingestion of a high amount of propafenone and a neuroleptic agent, with a suicidal legal-medical etiology.

Poster abstracts

P244. FATAL MEHADONE INTOXICATION IN AN INFANT

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Since the introduction of methadone maintenance programs in opiate dependence, fatal and non fatal accidental methadone intoxications in children have been reported by several authors. Often such intoxications occur in families in which parents or relatives are receiving methadone prescribed for opiate addiction.
In this report, we detail the deliberate and continual administration of methadone to an infant for the purpose of sedation, eventually resulting in the infant's death.
Peripheral blood from the femoral veins, blood from the right heart, urine, bile, gastric content, cerebrospinal fluid, hair and nails were collected for toxicological and biochemical analyses.
Methadone was detected in femoral blood, urine, gastric content, hair and nails. The concentration found in the femoral blood was within toxic and lethal levels. Hair and nail analysis confirmed methadone administration during the months preceding the death. Hair samples collected from the brother of the deceased also confirmed repeated methadone administration.
The cause of death was determined to be methadone intoxication and the manner of death was homicide.

P245. POSTMORTEM DISTRIBUTION OF FLECAINIDE IN A SUICIDAL OVERDOSE: A CASE REPORT

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Flecainide acetate is a very effective antiarrhythmic agent used in the treatment of ventricular arrhythmias. Post-mortem changes in blood drug concentration represent an important factor to be considered when evaluating the cause of death. As for Flecainide, its concentrations are reported to be 3.6-fold higher in post-mortem blood than in ante-mortem blood. In this case, ante-mortem blood was discarded and Flecainide analysis were performed in post-mortem blood and tissues; the high concentrations resulted could not have possibly been determined by post-mortem diffusion from gastric residue because the patient was given activated carbon ante-mortem.
In this case we studied the distribution of Flecainide in biological specimen, in order to exclude the post-mortem gastric diffusion because of the absorbing activity of carbon, and that blood concentrations added up to the amounts that was actually absorbed.. For this purpose Fleicainide was measured out in cardiac blood, peripheral blood, liver, bile, gastric residue and brain.
Flecainide acetate was obtained from MEDA PHARMA S.p.A, and Nalorfine HBr from S.A.L.A.R.S. S.p.A as the internal standard. Blood and tissue samples collected from autopsy were frozen at – 20° C until analyzed. Liquid and tissues were omogenated and extracted by solid-phase extractione (SPE) using Bond Elute Certify® (Varian Palo Alto, CA) cartridges. Quantification analysis were performed on Agilent GC7890-HP MSD5975 equipped with HP 5MS 5% capillary column. Four calibration curves were prepared in matrix (blood, brain, liver, bile) from 2 to 25 mg/mL for blood and from 2 to 25 mg/mL for tissues.
Flecainide concentration in post-mortem was: cardiac blood 10.16 mg/L, peripheral blood 8.64 mg/L, brain 4.19 mg/L, liver 59.6 mg/L, while in the bile it was 128 mg/L and gastric residue was negative. Two mechanisms of post-mortem have been reported to determine the increase in concentration of a drug: the passive diffusion from gastric residue, and post-mortem redistribution from organs containing elevated concentrations of the xenobiotic agent. In this case, the possibility of a post-mortem gastric diffusion of the drug appears unlikely because of the negative result in the gastric residue by GC/MS technique: in our opinion this fact is due to the absorption activity of the given activated carbon and the blood concentration is actually a consequence of absorbing metabolic activity before reanimation measures. Moreover, redistribution of Flecainide from tissues towards blood vessels is of little importance, without gastric redistribution. The circumstances surrounding the death pointed to a self-poisoning with Flecainide: the blood concentration of Flecainide was far over the range of toxicity, whereas the therapeutic dose of 200 mg corresponds to a plasma concentration of 214-281 µg/L (average 251) in healthy subjects, thus confirming a Fleicanide overdose.
In this suicidal case, an high peripheral blood concentration supported Fleicanide overdose as the only cause of death, excluding that toxicity depended on post-mortem gastric diffusion of the drug, because of the absorbing activity of the administered carbon at the recovery.

P246. THE ESTIMATION OF THE TIME-SINCE-DEATH USING THE POSTMORTEM CHOLESTEROL LEVELS IN MEDIAN NERVES.

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Forensic chemistry is useful in the estimation of post-mortem interval (PMI). Biochemical analysis of humor vitreous, cerebrospinal fluid, blood and urine can provide significant information in determining PMI; results are not univocal, limiting their general applicability.
The study was aimed to determine post-mortem interval by means of cholesterol (CHOL) level variations in peripheral nerve tissue at different times since death.
16 subjects (25-53 years old) without diagnosed neurological or metabolic diseases were chosen for the study.

During forensic autopsy, two samples of median nerves were collected from the wrist at intervals ranging from 30 minutes to two hours (t1 and t2); samples were collected from the right and/or left arm. Fragments of approximately 1 cm were collected and analyzed by gas chromatographic/mass spectrometry (GC/MS), after chemical digestion in basic environment. CHOL quantification in median nerve was performed by means of a calibration curve in the range (9.25-150) ng/µL. Data have been corrected with respect to the amount of nerve tissue analyzed and CHOL concentrations (in µg/mgtissue) were correlated to PMI (all samples were analyzed in triplicate and the mean values considered). The method was validated according to FDA guidelines and a statistical description of data performed.
Although different in absolute values, changes in CHOL concentrations over time were recorded in all subjects, depending on time-after-death, with respect to cholesterol basal levels reported in literature. In particular, an increment in cholesterol levels at t2 with respect to concentrations obtained at t1 recorded in all enrolled subjects. In order to eliminate inter-individual variations in cholesterol increment due to differences in collection times, concentrations were corrected with respect to dt (t2-t1=dt) and the variable CHOL_dt was subsequently divided into two sub-distributions according to the variable Time. A statistically significant difference in the two sub-distributions (p=0.028) was evidenced, confirming the actual increment in cholesterol concentrations over time. Data elaboration based on %ΔCHOL/dt (calculated as (CHOLt2-CHOLt1)/CHOLt2*100), within sampling time, dt = t2-t1), confirmed as cholesterol increment within sampling time positively correlates with post mortem interval. Data of cholesterol concentrations obtained for each subject at t1 and t2 have been correlated to time-after-death. As expected, differences in the interpolation curves are present, mainly attributable to inter-individual evolution of demyelinization process. Despite this, a similar trend was obtained for samples collected at similar PMI. Consequently, interpolation has been repeated after grouping subjects with respect to PMI, considering three groups: PMI<48 hrs, 48<PMI<78 hrs, PMI>78 hrs. Good results were obtained, especially for the first group (PMI<48 hrs) for which a correlation coefficient of 0.9362 was obtained and PMI can be calculated with an error ranging from -4 to 5.9 hrs. Mediane nerve tissue from the wrist shows a "relative" slow evolution of post-mortem phenomena and due to their peripheral position are not involved in putrefactive modifications typically observed after the death in thoraco-abdominal organs. Such regard together with the possibility of performing in each subject multiple nerve sampling at subsequent times-after-death make the proposed method useful for the estimation of PMI.

P247. DETECTION OF SYNTHETIC CANNABINOID 5-FLUORO ADB AND ITS POSSIBLE METABOLITES IN FOUR FATALITIES

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A designer drug was involved in four deaths within a relatively short period last year in Sendai, Japan. In each case, decedents possessed the same packet, labeled "heart shot black" which contained a dried plant with an aromatic scent. Autopsies revealed no specific findings in all cases.
We sought to identify the designer drug contained in the packages. Furthermore, we assayed the designer drug and its possible metabolites in the decedent's blood and urine to confirm drug use.
5-fluoro ADB (purity >98%) was purchased from Cayman chemical. The dried plants in the packages were treated with methanol, and the extracts were analyzed by gas chromatography-mass spectrometry (GC-MS, Agilent 5975C) and liquid chromatography (LC)-MS/MS (ABSCIEX QTRAP 5500). The drug was extracted from whole blood samples (external iliac venous blood) using the QuEChERS method and purified by dispersive solid phase extraction (SPE, C18, PSA, and MgSO4). Purified samples underwent LC-MS/MS. Compound-dependent multiple reaction monitoring-enhanced product ion (MRM-EPI) scanning in positive ion mode was used for the qualitative analysis, and the MRM mode was used for quantitative analysis (transitions were: 378-318, 378-233). Urine samples (non-hydrolyzed) were deproteinized by adding methanol and centrifuged. The supernatant was evaporated and redissolved in pure water. The solution was analyzed by LC-MS/MS for detecting 5-fluoro ADB and by LC-quadrupole time-of-flight (QTOF, AB SCIEX Triple TOF 5600) for searching metabolites.
All packages of "heart shot black" contained a cannabinoid-type drug with a mass spectrum identical to 5-fluoro ADB, which was restricted by the Pharmaceutical Affairs Act in Japan. 5-Fluoro ADB was detected in all four decedent's blood at concentrations below 1 ng/mL (estimated concentration). LC-QTOF results revealed the presence of possible 5-fluoro ADB metabolites in the urine of the decedents. These metabolites were generated by methyl ester hydrolysis, defluorination, carboxylation, and hydroxylation of the parent drug.
We confirmed that the synthetic cannabinoid 5-fluoro ADB was present in the packages. We also detected 5-fluoro ADB and its possible metabolites in whole blood and urine samples, respectively. These metabolites may be generated in a manner similar to other indole-based synthetic cannabinoids.

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P248. POSTMORTEM BIOCHEMICAL INVESTIGATIONS IN SUSPECTED STARVATION-INDUCED KETOACIDOSIS

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Apart from diabetes mellitus and infrequent cases of intoxication (salicylate, isoniazid and isopropyl alcohol poisonings), some nutritional conditions may also be responsible for ketoacidosis, such as starvation-induced ketoacidosis, alcoholic ketoacidosis and ketogenic diet-induced ketoacidosis. Starvation-induced ketosis involves carbohydrate depletion with free fatty acid mobilization. The resulting ketosis is generally mild and not life threatening. Hence, starvation-induced ketoacidosis is uncommonly resported as the cause of death in the forensic literature.
In this study, we present 4 cases of suspected starvation-induced ketoacidosis, in which postmortem investigations allowed traditional causes of ketoacidosis to be excluded.
Body mass index ranged from 17 to 22. Evidence of starvation at external examination and autopsy was noticed in one out of 4 cases. Postmortem investigations included conventional autopsy, histology, toxicology and postmortem biochemistry (acetone and beta-hydroxybutyrate determination as well as prealbumin and thyroid hormone measurement). Acetone levels were determined by the use of headspace gas chromatography with flame ionization detection. Beta-hydroxybutyrate concentrations were measured using an an enzymatic photometric method. Prealbumin values were determined using an automated immunoturbidimetric method.
In all presented cases, blood acetone (normal levels : < 10 mg/l) and beta-hydroxybutyrate (normal levels: 50 – 170 µmol/l) concentrations were markedly increased. Prealbumin concentrations (normal levels 0.20 - 0.40 g/l) were decreased, thus corroborating the hypothesis of inadequate nutrient intake. Blood acetone and beta-hydroxybutyrate concentrations in some cases were within levels that are considered significantly elevated (> 90 mg/l and > 2500 µmol/l, respectively, according to the available medico-legal literature).
Severe, life-threatening ketoacidosis is possible when starvation is complicated by a stressful event or extraordinary conditions. Starvation-induced ketoacidosis should be suspected in presence of markedly increased blood acetone and beta-hydroxybutyrate concentrations, decreased blood prealbumin levels and exclusion of alternative causes of ketoacidosis.

P249. POST MORTEM DISTRIBUTION OF CYANIDES: DIFFICULTIES OF INTERPRETATION

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Cyanide concentrations in blood specimens can be very different depending on the type of post mortem samples. In the case of the death of a 53-year old man (grenade explosion) followed by a post mortem fire, we observed unexpected free cyanide concentrations, particularly in terms of distribution: 317µg/L in the cardiac blood and 1100µg/L in the peripheral blood; as the carboxyhemoglobin (COHb) were 13.4 and 9.5% respectively.
These results led us to conduct a retrospective study on the distribution between cardiac and peripheral blood concentrations of cyanide and COHb rate.
The cases included in the study were autopsies performed at Grenoble forensic institute between 2012 and 2014 for which cardiac and peripheral blood samples were available and for which COHb were higher than 10% or cyanide concentrations above 200µg/L. COHb determination was performed by an automated method (CO-oximeter) or manually by spectrophotometry. Cyanides were quantified using a liquid chromatography-tandem mass spectrometry method. In addition, a scale (from 0 (lowest risk) to 4 (high risk)) was generated to estimate body injuries which potentially exposed blood to the atmosphere.
16 cases were included in this study. The obtained HbCO levels varied from 2.6 to 75% in the peripheral blood and from 3.9 to 87.8% in cardiac blood where the median and mean were comparable. Rate of COHb between cardiac and peripheral blood ratios were between 0.97 and 2.54, with a median value=1.20 and an average=1.32. The correlation between the levels of COHb according to the blood collection site was suitable (r=0.96). The cyanide concentrations ranges were 74 to 3050µg/L in the peripheral blood (median=577µg/L and average=851µg/L) and 108 to 3430µg/L in the heart blood (median=625µg/L and mean=823µg/L). The ratios cyanide rate between cardiac and peripheral blood were between 0.11 and 3.07 with a median=1.02 and an average=1.54. The correlation between cardiac and peripheral blood cyanide concentrations according to the sampling site was low (r=0.77) due to a very large values dispersions. Indeed, the cardiac/peripheral blood cyanide ratio was close to 1 (0.8-1.2), lower (0.1-0.7) and higher (1.3-6.1) for each tertile measurements.
The correlation between the levels of COHb in heart blood and peripheral blood was demonstrated whereas the results were scattered for cyanide. In addition to conservation troubles in biological matrix (instability, artifactual production by cyanogenic bacteria...) well described for cyanide, another hypothesis described in the literature, like a post-mortem fixation phenomenon from atmosphere hydrogen cyanid and blood directly exposed from body injuries. This hypothesis was retained in the introduction presented case as the wounds were scaled at the level 4.

P250. QUALITATIVE ANALYSIS OF PSILOCIN AND α-AMANITIN IN URINE BY UPLC-MS/MS: METHOD DEVELOPMENT AND VALIDATION

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Mycotoxin intoxications are a rare but severe intoxication with serious consequences for the affected individuals. The exposure to these toxins may have different etiologies, mainly: accidental exposure by ingestion of poisonous mushroom species, or intentional exposure by ingestion of mushrooms with hallucinogenic properties, also called "magic mushrooms". Accidental poisoning can result from the ingestion of Amanita species such as Amanita phalloides, which contains α-amanitin and β-amanitin as the major lethal toxins. Psilocin and psilocybin are the most common psychoactive substances of the hallucinogenic mushrooms; they are used as a recreational drug and can be found in mushrooms of the genera Psilocybe, Panaeolus, Conocybe and Gymnopilus.
To detect and confirm the presence of these substances in biological samples the authors developed and validated an analytical method that allows qualitative confirmation of α-amanitin and psilocin by UPLC®-MS/MS in urine. Psilocybin is converted into psilocin by the human body and excreted through the urine without processing and is thus indirectly included in this determination.
Urine samples (0,5 mL) spiked with internal standard (psilocin-d10) were submitted to a solid phase extraction (HLB Oasis®, 3cc, 60mg) and analyzed by UPLC®-MS/MS. Chromatographic separation was possible in a 6 min analytical run time, using a UPLC® ACQUITY BEH C18 1,7µm [2,1 x 100 mm] column and a gradient mobile phase consisting of acetonitrile and 0,1% formic acid (flow 0,5mL/min). The detection of the analytes was performed with a mass detector Acquity ™ TQD tandem - quadrupole MS fitted with a source in positive electrospray ionization mode (ESI). Analytes confirmation was achieved using two MRM (multiple reaction monitoring) transitions for each target compound: m/z 205>58 and 205>160 for psilocin, m/z 919,48>259,13 and 919,48>901,53 for α-amanitin and m/z 215>66 for psilocin-d10.
Validation procedures showed that the analytical methodology proposed is sensitive (LOD= 2,5ng/mL for psilocin and α-amanitin based on signal-to-noise ratio 3:1) and selective (0% false positives and <10% of false negatives for both substances evaluated by analysis of 20 blank urine samples). The extraction efficiency was studied at two different concentrations (50 and 200ng/mL) and the results were 36% and 58% for psilocin, 89% and 99% for α-amanitin respectively. No carryover between analyses was observed. This method meets the analytical validation parameters usually accepted internationally and adopted by the Chemical and Toxicology Forensic Laboratory (Portugal) for qualitative analysis methods in terms of selectivity, sensitivity and extraction efficiency.
The authors developed and validated a qualitative analysis method for the confirmation of psilocin and α-amanitin in urine by UPLC®-MS/MS. These substances are typically involved in cases of poisoning by mushrooms, allowing the forensic laboratory to respond to specific requests concerning the investigation of mycotoxins intoxication cases.

P251. DETERMINATION OF COCAINE ANALYTES IN BIOLOGICAL SPECIMENS BETWEEN 2010 AND 2014

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In recent years, despite the efforts made throughout Europe to decrease the consumption of illicit substances and alcohol, this is still one of the major causes of mortality among young people in this continent, both directly, through overdose (drug-induced deaths), and indirectly, through drug-related diseases, accidents, violence and suicide. The consumption of amphetamines, cannabis, benzodiazepines, heroin and cocaine is associated with an increasing risk to get involved or be responsible for an accident, and this danger expands when the drug is combined with other psychoactive substances such as alcohol. Recent studies revealed that among the Portuguese drivers there is a significant prevalence of cases with positive results for alcohol and/or other drugs including anxiolytic medicines.
The aim of this study was to assess the trends of concomitant consumption of cocaine and ethanol in the Centre of Portugal during the last 4 years.
It was carried out a retrospective investigation of positive toxicological analyses for drugs of abuse and ethanol performed during the years 2010 and 2014 in the Service of Chemical and Forensics Toxicology of the Centre Branch of the National Institute of Legal Medicine and Forensic Sciences. To analyse cocaine, benzoylecgonine, ecgonine methyl esther, cocaethylrne and norcocaine it was used a validated methodology in blood, which adopts a mixed-mode solid phase extraction procedure. Following, a microwave fast derivatization of the dried extracts was performed and these were finally analysed on a gas chromatography-mass spectrometry (GC-MS-EI) operated in selected ion monitoring mode (SIM). Ethanol determinations were made by a validated procedure using headspace gas chromatography-flame ionization detection (GC-FID-HS).
A total of 120 blood samples were positive for cocaine (10 - 6942 ng/mL), benzoylecgonine (35 - 16797 ng/mL), ecgonine methyl esther (20 - 14863 ng/mL) and, in a singular case, norcocaine (160 ng/mL). Out of these, 35 cases with significant concentrations of ethanol (which ranged from 0.14 to 1.2 g/L) were identified, 15% of which had concentrations of cocaethylene (which ranged from 5 to 200 ng/mL). There were also 24 cases where other substances (such as cannabinoids, morphine, benzodiazepines, tramadol, paracetamol and lidocaine) were found. It was also possible to verify that 77% of the samples belonged to men aged between 24 and 47 years old and 23% to women aged between 27 and 36 years old.
Although consumption of cocaine in Portugal has not increased throughout the last 4 years, the data obtained from samples collected in the centre area of Portugal is still of concern since it was achieved mostly from road accidents. It is, therefore, possible to conclude that there has been a concomitant intake of more than a single drug.

Poster abstracts

P252. DEGRADATION OF 4-METHYLMETHCATHINONE BY PUTREFYING BACTERIA

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Drugs in decomposed post-mortem matrices may undergo unique biotransformation by putrefactive bacteria. Understanding the metabolism of drugs by these microorganisms is therefore important for drug detection in a putrefied biological matrix. However current knowledge on putrefaction-mediated degradation of drugs is significantly lacking. It has been previously shown that 4-methylmethcathinone (4MMC) is unstable and tends to degrade in post-mortem matrices. 4MMC was thus chosen for proof-of-concept investigation into its biotransformation pathways during putrefaction. More specifically the study aimed to investigate the degradation of 4MMC by various putrefactive bacteria and to elucidate the possible structures of formed degradation products which may be suitable for drug monitoring purposes in post-mortem toxicology.
Four putrefactive bacteria were used in this study, i.e. Staphyloccocus aureus, Escherichia coli, Kliebsiella pnuemoniae and Proteus vulgaris. Bacteria were grown in Oxoid CM1135 brain heart infusion broth. After adding 4MMC, the culture was incubated at 37°C for 24, 48 or 72 h. Following removal of bacteria by filtration, the culture media was basified and extracted with diethylether. The extract was analyzed on an Agilent Technologies 1290 Infinity liquid chromatography (LC) system coupled to either an Agilent 6490 triple quadrupole mass spectrometer or an Agilent 6510 accurate mass quadrupole time-of-flight mass spectrometer. LC was performed on a Poroshell 120 EC-C18 column (2.1 mm x 75 mm, 2.7 µm). Electrospray ionization was performed in positive ionization mode and collision-induced dissociation (CID) experiments were conducted using collision energies ranging from 5-40 eV.
All four putrefactive bacteria were capable of degrading 4MMC extensively under the experimental conditions explored. Many degradation products observed were considered to be specific to the putrefactive microorganism used. Identification of bacteria-mediated degradation products of 4MMC was based on careful comparison of the obtained high resolution mass spectrometric data with those from the medium control, bacterial control and drug control. Of particular interest was the discovery of a novel degradation product common to all four bacterial species. The product was tentatively assigned as 2-hydroxy-(4-methylphenyl)propan-1-one. This assignment was supported by the observed protonated molecule $[M+H]^+$ at m/z 165.0910 ($C_{10}H_{13}O_2$, calc. m/z 165.0911) together with two major product ions at m/z 147.0802 ($C_{10}H_{11}O$, calc. m/z 147.0804) and m/z 119.0852 (C_9H_{11}, calc. m/z 119.0855).
4MMC is susceptible to degradation by putrefactive bacteria. Some of the identified degradation products, such as 2-hydroxy-(4-methylphenyl)propan-1-one, may serve as useful indicators of 4MMC in decomposed post-mortem specimens.

P253. LETHAL INTOXICATIONS OF ANIMALS IN GREECE DURING THE YEARS 2012-2014

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The poisoning of animals is a criminal offence that is punished by imprisonment and fine according to the Greek laws. Intoxications of domestic and company animals are quite frequent in Greece and most of them are lethal. These intoxications sometimes occur after accidental exposure through water or food, but there is a great number of cases where poisoning is intentional through baits. The most frequent responsible agents for these poisonings are pesticides, like carbamate or organophosphate esters, chlorinated pesticides, cyanides, and anticoagulant rodenticides.
Since the poisoning of animals is a criminal act, the forensic investigation of these cases is undertaken by the Department of Forensic Medicine and Toxicology of the University of Athens, in collaboration with the Ministry of Reconstruction of Production. A significant number of poisoning cases have been recorded in Greece during the years 2012-2014. The aim of this study is to present these investigated lethal animal intoxications, the kind of animals exposed and the pesticides detected in each case.
An in-house developed GC-MS method for the determination of pesticides in biological specimens was used during the toxicological investigation of intoxication animal cases. The method is simple, rapid, specific, sensitive and using only 500 µL of biological specimen (blood and/or stomach content) or material evidence (food, baits, water etc.), that was liquid-liquid extracted with a mixture of toluene: chloroform (4:1, v/v) prior to the GC-MS analysis.
716 cases of animal lethal poisonings were investigated by the Department of Forensic Medicine and Toxicology and by the Ministry of Reconstruction of Production, during the years 2012-2014. The cases are classified according to the kind of the poisoned animal and the kind of the detected poison. A different classification is made for baits according to the kind of the material used and the kind of the detected poison. The most often poisoned animals were dogs, cats, cattle and bees. A significant number of poisonings of wild animals were also recorded. The most frequently detected poisons in biological samples and baits were methomyl, carbofuran, endosulfan, phorate, omethoate, methamidophos, chlorpyrifos, terbufos, oxamyl, and cyanides.
Pesticides are used extensively worldwide, therefore, they could be the cause of animal accidental or intentional poisonings, fatal or not. The occurrence of pesticides poisoning in animals is of special concern in agricultural areas like Greece. This survey shows that in Greece the deliberate misuse of substances to kill animals is very common and still far from being eradicated. At the moment a scheme to fight the poisoned baits phenomenon is still far from being effective. Elaboration of a complex strategy, involving authorities, as well as veterinarians and citizens, is the first step to defeat this harmful practice. Special caution should be taken during the application of pesticides in order to avoid accidental animal poisonings.

P254. **QUETIAPINE-RELATED DEATHS: A WORRYING INCREASE**

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Quetiapine is a dibenzothiazepine antipsychotic agent that was approved in 1997 by the US Food and Drug Administration for the treatment of psychotic disorders. Quetiapine, commonly marketed as Seroquel® (AstraZeneca), is used to treat the symptoms of schizophrenia, bipolar disorder and major depressive disorder. There is also evidence that quetiapine may benefit patients diagnosed with a mental illness who are also dependent on cocaine, amphetamines, or both, though more rigorous studies are needed. During the last years, quetiapine has also been associated with case reports of patient misuse and abuse and there are indications that it possesses an addictive potential. Curiously, the drug accused of misuse/abuse is used as a treatment for those who misuse and abuse various substances.
During the previous years, the number of forensic cases in Greece where quetiapine was detected was ranging from 5 to 10 cases per year. During the last year, the number of deaths related to quetiapine was dramatically increased. The aim of this study is the determination of quetiapine concentrations in post-mortem blood samples during the toxicological investigation of forensic cases in order to identify the contribution of quetiapine to the cause of the death.
A GC/MS method for the determination of quetiapine in post-mortem blood samples was developed and validated according to international guidelines. The developed method includes solid-phase extraction of blood samples using HF Bond Elut C18 (Agilent Technologies, USA) followed by silylation using N-methyl-N-tert-butyldimethylsilyl-trifluoroacetamide (MTBSTFA). This method was successfully applied in authentic post-mortem blood samples during the toxicological investigation of forensic cases, where metabolites of quetiapine were detected during general unknown screening of urine samples.
Twenty-eight cases were investigated and the results were classified according to:
- the sex of the victims (male: 19, female: 9);
- the cause of death (poisoning: 9, pathological causes: 15, asphyxiation: 3, multiple injuries: 1);
- the manner of death (suicide: 11, sudden death: 14, accidental death: 3);
- the presence of alcohol (6 cases) or other drugs (28 cases);
- the blood quetiapine concentration (5.2-14112 ng/mL).

Quetiapine was determined at toxic levels (>1800 ng/mL) in six cases and at lower concentrations (5.2-1160 ng/mL) in the other twenty-two cases.
There are growing concerns worldwide about the misuse and abuse of quetiapine and more studies are needed to clarify its contribution to the cause of death. The pharmacological theories to explain potential risk remain unsubstantiated as there are no available relative animal or human studies. It is remarkable to be mentioned that in all presented cases one or more drugs, except from quetiapine, were also detected.
Quetiapine is being found with increasing frequency in post-mortem cases in Greece. Physicians should be alert about the toxicity of quetiapine and its possible misuse or abuse, especially in patients under multiple drug therapy or drug addicts.

P255. **BODYPACKING IN GREECE. IT STILL EXISTS.**

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Greece holds a key geographical position in the area of South-East Europe concerning drug smuggling, having extensive borderline with non-EU countries, 2.500 islands, several international harbours and airports and traversed also by the «Balkan route» as a gate of entrance to the Western European market. Bodypacking, transportation of moderate amounts of illicit drugs by internal concealment, is a worldwide phenomenon that is not unknown in Greece and is still used by smugglers. "Bodypackers" or "mules" are people who illegally carry illicit drug-filled packages into a body cavity, in order to across borders and transport drugs without being detected. The "egg" packages are made of various materials, latex, condoms, toy balloons or aluminum foil and they are swallowed or inserted in the mouth, rectum or vagina. Cocaine is one of the most trafficked drugs, followed by heroin. The most frequent cause of death among bodypackers is due to overdoses resulting of leaking or opening of drug packages within the stomach and/or gastrointestinal tract.
The aim of this presentation is to present characteristic bodypacking cases that appeared in Greece the last five years and to emphasize that bodypacking is a method of transportation of drugs that still exists.
Characteristic cases from the Departments of Forensic Medicine and Toxicology in Athens and Thessaloniki as well as from the General Chemical State Laboratory were selected and presented along with the toxicological findings in each case.
- 7 men coming from Sao Paolo, Brazil were arrested by Athens Airport Police when they were X-rayed after information given to the custom authorities that they were carrying cocaine in their bowel. Laxatives were administered and 583 packages were recovered containing 11.506g of cocaine.
- 1 man coming from Montevideo, Uruguay, was arrested carrying in his stomach 40 "eggs" containing 1084g of heroin.
- A Nigerian and a Peruvian were arrested by Athens Airport Police and after whole irrigation with polyethylene glycol the Nigerian passed 54 machine-wrapped packets per rectum while the Peruvian passed 20 handmade packets

containing cocaine.
• 1 man was found dead and autopsy revealed in his gastrointestinal tract 55 "eggs" containing 800g of heroin. 1.45µg/ml of morphine was detected in his blood.
• A 46-year-old Greek woman was carrying in her digestive tract 2 plastic wrapped packages each containing about 10g of cocaine powder. The bodypacker died by acute cocaine intoxication due to inflation and rupture of both packages in her stomach, after her arrival in Rhodes island from Turkey. Cocaine (5.46±0.23µg/mL), benzoylecgonine (11.97±0.42µg/mL) and ecgonine methylester (10.71±0.59µg/mL) were found in her blood.
Bodypacking is still a practice for drug smuggling, despite the extremely high hazards for the bodypacker. Single or multiple package rupture of the packages that are transported by bodypackers may lead to sub-lethal or lethal release of their content. On the other hand, effective packaging protects the smuggler from substance release but consequently abolishes forensic findings. All implicated authorities should be extremely suspicious of any sudden, unexpected illness or death among individuals who have recently travelled from destinations involved in drug trafficking.

P256. VITREOUS HUMOR AS AN ALTERNATIVE OR COMPLEMENTARY SAMPLE IN DRUG OF ABUSE SCREENING BY ENZIMATIC IMMUNOASSAY

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Rapid screening tests and small sample volumes are essential for an efficient performance of laboratories carrying out a high number of forensic examinations. The use of immunoenzymatic methods for the analysis of drugs of abuse and/or other groups of substances including medicines is common in laboratories that examine a large number of samples, which results in a significant reduction in analysis time and cost. The analysis of blood samples has numerous advantages, the main one being the possibility of correlating the levels found for a xenobiotic with the described effects or symptoms. However, the vitreous humor is sometimes the best available sample in postmortem analysis, for example, in cases of severely burned, decomposed or mutilated bodies. Vitreous humor may also be a complementary sample in cases of sample degradation since it contains few cells and is rarely subject to bacterial growth, being less affected by postmortem changes.
The objective of this study is to evaluate the applicability of vitreous humor as an alternative specimen in the absence of blood and urine or as a complementary sample, using the same validated enzyme immunoassays methods applied for whole blood to analyze drugs of abuse.
Immunoassays were performed on an automated EIA analyzer Coda (BioRad), using the same protocols as for whole blood samples. The results were evaluated according to the set cut-off values for blood samples: Opiates - 50 ng/ml of morphine; Cocaine metabolites - 50 ng/ml of benzoylecgonine; Cannabinoids - 50 ng/ml of 11-nor-carboxy-delta-9-tetrahydrocannabinol; Amphetamines - 100 ng/ml of DL-amphetamine and Methamphetamines - 100 ng/ml of DL-methamphetamine. Vitreous humor specimens [N=90] were collected during forensic autopsies as part of the routine protocol adopted by the Centre Branch of the National Institute of Legal Medicine and Forensic Science. The screening results obtained for vitreous humor were evaluated by comparison with those obtained for blood samples using immunoassay and GC/MS techniques.
The results obtained with vitreous humor were in agreement with those resulting from blood samples analysis, with an exception for the substances included in the group of cannabinoids.
This study shows the applicability of enzyme immunoassays in the analysis of drugs of abuse in vitreous humor using the same protocols as for whole blood, so providing a useful alternative for the screening of drugs of abuse in very rotten blood samples or in the absence of blood and/or urine. The method showed to be particularly effective for the screening of opiates and cocaine metabolites. Regarding the screening of amphetamine and methamphetamine groups the method showed a total absence of false positive results, considering those obtained by GC-MS, in contrast with a significant number of false positive screening results obtained with degraded blood samples from same cases.Regarding cannabinoids it was found that when compared with the results obtained for whole blood this matrix is not suitable to the study of this group of substances.

P257. INTERPRETING RESULTS OF ETHANOL, CARBAMAZEPINE AND TOPIRAMATE IN PUTREFIED POSTMORTEM SPECIMENS: A CASE REPORT

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Carbamazepine (CBZ), Topiramate and Alcohol, were detected in 37 years old woman founded eighteen months after his death, wrapped in plastic bags and blankets and buried at home hidden in the wall. The body was in an advanced state of saponification. The woman was in treatment with anticonvulsant drugs and she often drank alcoholic beverages. The cause of death was a strangulation investigated by immunohistochemical with glycophorin A (GPA) on the skin of the neck and histological on the lungs. Toxicological investigations have allowed to rule out that the woman was in an alteration psychophysical for alcoholic or drug abuse; thus has been further refined the causal diagnosis of death, ascribed to dynamic murder.
The methods applied on the body putrefied gave results in accord to the dynamics of the events that led to the death of the woman, as it was found at the end of the first instance, which culminated in the murder conviction of the accused. Carbamazepine, Topiramate and Alcohol were quantitated in abdominal effusion, gastric wall, spleen,

fluid in douglas, skeletal muscles, endothoracic fluid, kidney, liver, heart and bone marrow. The forensic interest of performing quantitative analysis in all these samples is due to the absence of toxicological data in the recent scientific literature. The results are reinforcing the toxicological data obtained on skeletal muscle. In conclusion, we believe that the values found can be used as a comparison in similar cases.
CBZ, 10-11epoxide-CBZ and Topiramate was recovered in samples deproteinized by acetonitrile spiked with dansyl-norvaline 100 µM as internal standard. Compounds were separated on a reversed-phase High-Performance Liquid Chromatography and samples concentration were measured by a triple quadrupole tandem mass spectrometer, set up in positive mode and multiple reaction monitoring (MRM). Alcohol was detected in any specimens detected by HS-GC/FID with iso-propanol as internal standard.
CBZ concentration were 0.93, 2.25, 4.48, 2.62, 0.69, 13.6, 2.64, 0.49, 4.37, 2.93 µg/g ; Epox-CBZ concentration were 1.0, 0.64, 0.91, 1.13, 0.46, 0.99, 1.1, 0.67, 0.7, 0.51 µg/g; Topiramate 0.92, 0.11, 0.35, 0.83, 0.21, 1.23, 0.68, 0.64, 0.95, 0.85 µg/g; Alcohol 0.35, 0.38, 0.34, 0.75, 0.41, 0.38, 0.28, 0.34, 0.22, 0.17 mg/g.
To our knowledge this is the first report of the presence of carbamazepine, topiramate, and alcohol in post mortem putrefied specimens.

P258. CONCENTRATIONS OF R/S-METHADONE AND R/S-EDDP IN POSTMORTEM BLOOD AND HAIR USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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Methadone (MTD) is a long-acting opioid widely used for the treatment of opioid-dependence. The pharmacokinetics of MTD is stereoselective; hence it may be relevant to distinguish between the two enantiomers for the interpretation of postmortem MTD concentrations.
The aim of this study was to develop a chiral HPLC-MS/MS method for the determination of MTD and the main metabolite EDDP in postmortem blood and hair. The method was used to evaluate the R/S-ratios of MTD and EDDP in fifteen MTD poisoning case. All subjects included in the study had participated in methadone treatment maintenance (MMT) program, and the cause of death was attributed to MTD poisoning.
Blood samples were protein precipitated with acetonitrile followed by solid-phase extraction. Hair samples were aligned and cut into three segments (S1: 5; S2: 10 and S3: 10 mm). Each hair sample (10 mg) was washed and pulverized followed by overnight incubation in a solvent mixture of methanol, acetonitrile and ammonium format buffer (25:25:50). The enantiomers of MTD and EDDP were fully separated on a chiral α(1)-acid glycoprotein column using a gradient of 15 mM ammonium acetate buffer (pH 5.3) and 2-propanol. The detection was performed using positive electrospray ionization (ESI+) and multiple reaction monitoring (MRM) acquisition mode. The method was fully validated according to international guidelines.
In blood, the R-and S-MTD concentrations ranged from 0.21 to 2.0 (median: 0.65) and 0.14 to 1.0 mg/kg (median: 0.27), respectively. In hair, the concentrations of R/S-MTD were similar in all three segments, indicating that MTD was administered continuously in the last 2.5 months prior to death. In the most recent segment (S1), the R-and S-MTD concentrations ranged from 2.4 to 39 (median: 16) and 1.4 to 24 ng/mg (median: 7.3), respectively, which is higher than the MTD concentrations reported in living MMT patients. Thus in both matrices, a higher concentration of R-MTD was found compared to S-MTD with R/S-ratios ranging from 1.5 to 2.4 (median: 2.0) and 1.5 to 2.6 (median: 1.7) in blood and hair, respectively. The corresponding median R/S-ratios of EDDP were 0.79 and 0.96, which is consistent with the fact that the metabolism of R-MTD to R-EDDP is slower than for the S-enantiomer. The R/S-ratios of MTD found in postmortem blood were slightly higher than R/S-ratios reported in plasma from living MMT patients, while the R/S-ratios of MTD in hair were similar to R/S-ratios reported in living MMT patients.
A chiral method was well established and successfully applied to blood and hair samples from fifteen deceased MMT patients. A dominance of R-MTD was found in both blood and hair, indicating that R-MTD was accumulated in blood due the longer half-life compared to S-MTD and hence a larger amount of R-MTD was incorporated into the hair than S-MTD.

P259. REPEATED ATTEMPTED MURDER BY POISONING: ABOUT AN ORIGINAL CASE

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A case of repeated attempted murder by administration of several psychoactive drugs is presented. Such cases are reported rarely. A 62-year-old man was hospitalized in Intensive Care Unit (ICU) after aspiration pneumonia secondary to undetermined neurological disease. He presented an unexplained loss of consciousness during a visit from his wife (H = 0). The nurses noted that the central venous catheter had been manually clamped, and contained a viscous white liquid instead of the prescribed ordinary saline solution. The hospital director informed the police. Blood samples were taken immediately; infusion tubing and bubble trap were seized. Forty-eight hours later (H = 48), the police officers apprehended his wife pouring a liquid into his fruit salad. A flask labelled «CYAMEMAZINE 40 mg/mL», a dropper pipette and a syringe were seized from her. Cyamemazine is a phenothiazine derivate indicated in the treatment of bipolar disorders and schizophrenia. The fruit salad and its container were also seized. In her car, a bottle of hydrochloric acid was found (not seized). A sample of the victim's hair was taken
This case report of a repeated attempted murder is presented to demonstrate the considerable forensic importance of the analytical procedures in the diagnosis of poisoning.
Serum screenings were done by immunoassay, GC-MS and LC-DAD. Benzodiazepines, antihistamines, antipsychotic

drugs, vasodilators and antiulcer agents were analyzed in serum by GC-MS and LC-DAD. Three 6-cm long, brown hair locks were cut in 3 segments of 2 cm each and analyzed by GC-MS/MS. Non-biological samples were analyzed following the same procedures and completed by pH measurements and tests for chloride ions.
Analyses in serum showed, beside the prescribed drugs in ICU, the presence of cyamemazine (0.14 µg/mL) and hydroxyzine (0.07 µg/mL). Cyamemazine was also identified in the infusion tubing and the bubble trap and was quantified at concentrations of 0.67 µg/mL in the dropper pipette and 42 mg/mL in the flask, respectively. In the fruit salad and its container, the measured pH was 1.0 and chloride ions were identified (not quantified). The segmental results of hair testing (0 – 2/2 – 4/4 – 6 cm) are as follows (pg/mg): cyamemazine: 9960/1610/2367, haloperidol: 9200/1391/227, hydroxyzine: 280/173/163, cetirizine: 25/12/26, amitriptyline: 7450/1850/3260, venlafaxine: 332/560/260. Cyamemazine concentrations are in favor of a very high and regular consumption during the previous 6 months, those of the other drugs in favor of a regular consumption in doses compatible with a therapeutic use.
The analyses of biological and non-biological samples led to the documentation of the attempted murder in ICU. The hair analysis and the data of the victim's medical records together permitted us (1) to establish that his wife administered to him without his knowledge several drugs over months, and (2) to understand the origin of his symptoms.

P260. THE PREVALENCE AND REDISTRIBUTION OF ISO-ALPHA-ACIDS IN POSTMORTEM CASEWORK THAT CONFIRM BEER CONSUMPTION

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Iso-α-acids (IAA) derived from the hop plant (Humulus lupulus L.), and three structurally similar but chemically-altered IAA known as "reduced IAA" (rho-, tetrahydro- and hexahydro-IAA), are beer-specific ingredient congeners found in beer. These bittering products can be used specifically in brown, green or clear bottled beer. A protein precipitation extraction and UHPLC-MS/MS method was validated for detection of these congeners in biological specimens to confirm beer consumption. Pharmacokinetic studies showed the IAA groups were bioavailable for hours after beer consumption in simulated drinking studies.
To determine the feasibility of detecting IAA groups in postmortem specimens and investigate the prevalence of beer consumption confirmations and the postmortem redistribution (PMR) of IAAs in coronial casework.
The IAA concentrations of mortuary admission and autopsy whole blood, serum, urine and vitreous humour postmortem specimens from 130 analysed coronial cases were investigated and compared with the case circumstances. Iso-α-acids were able to be detected in postmortem specimens. Of the 50 coronial cases where beer was mentioned in the circumstances, 87% had one or more IAAs detected. In cases that only had a positive BAC greater than 0.01 g/dL (n = 60), 57% of these cases detected IAAs and therefore suggested that beer may be at least partly responsible for an elevated blood alcohol. Where neither beer was mentioned nor a BAC recorded, no IAAs were detected. An association was observed with the length of time from death to collection of specimens, and PMR. Several IAA groups had a serum to blood ratio of ~3, and a weak association between BAC and IAA groups was demonstrated. Vitreous humour and urine specimens contained low concentrations and prevalence of IAA groups.
This alcohol congener analysis technique is a suitable procedure to confirm, and reveal, beer consumption in a range of typical coronial postmortem specimens and casework. The detection of reduced IAA type congeners may suggest consumption of green and/or clear bottled beer ingestion. This study shows that although beer consumption can be demonstrated, caution is required when comparing posrtmortem IAA levels to BAC.

P261. ENTOMOTOXICOLOGY: LARVAE ANALYSIS IN A REAL CASE OF HOMICIDE/SUICIDE

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A 40 year-old female and her children (a boy and a girl) were found completely burned by fire in a car. Video footage taken from home CCTV cameras showed that the mother administered an unknown liquid (subsequently identified as a drink containing diazepam) to the children. During post-mortem examination (performed 4 hours after bodies recovery) fly larvae belonging to Calliphoridae (Lucilia sericata) and Sarcophagidae (Sarcophaga sp.) species were collected.
The aim of this work was to verify the intake of diazepam by the children through the analyses of larvae collected from burned bodies.
A validated method for the determination of diazepam and its metabolites was used for toxicological analyses, using spiked larvae samples with the benzodiazepines. In detail, frozen larvae (about 200 mg) were repeatedly washed to remove any external contamination and homogenized by Precellys®, a homogenizer produced by Bertin-Technologies, where the sample is placed in a tube containing ceramic beads and 2 ml of phosphate buffer with deuterated internal standard (flunitrazepam-d7). A rapid shaking of vials causes the lysis of the larvae. Then 2 ml of hexane were added to the solution. After mixing and centrifugation, the supernatant was discarded and 0.5 ml of saturated Na_2CO_3 were added to the solution. After mixing and centrifugation the acetonitrile was collected in a clean tube and derivatized with 50 µl BSTFA. The analyses were performed by GC/MSMS.
The results showed that the larvae samples collected from the children were positive for diazepam and its metabolites, whereas the larvae collected from the mother's body were negative for diazepam and metabolites. The larvae collected from the daughter were positive for diazepam, nordiazepam and oxazepam at concentrations of 9.1, 4.4

and 7.8 ng/g, respectively. The benzodiazepines observed in the son were much higher, with concentrations of 71.5, 43.1 and 46.3 ng/g, respectively for diazepam, nordiazepam and oxazepam.
These results are in agreement with the data obtained by analyzing the cadaveric tissues, in fact, in the latters the concentration of benzodiazepines were higher in the son than in the daughter. This case shows that entomotoxicology can be a useful tool for the determination of drugs in burned corpses. To our knowledge this is the first case of drug determination from larvae growth on burned cadavers. In this case the toxicological analysis of the larvae confirmed the presence of diazepam in the children, despite having little organic material on which to perform the analysis.

P262. TWO FATAL POLYINTOXICATION CASES INVOLVING CITALOPRAM

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Citalopram is a selective serotonin reuptake inhibitor (SSRI) class of antidepressants prescribed to treat severe depression and treat anxiety. Marketed in many countries, it is easily available to the French population, known to be one of the biggest consumers of antidepressants in Europe. Although citalopram overdose has long been known to cause cardiotoxicity especially QT prolongation and torsades de pointes, seizures and serotoninergic syndrome, only a few reports have analysed citalopram levels in post-mortem blood.
We report two fatal cases involving citalopram: a 47 years old woman and a 55 year old man. Both treated for severe depression, were discovered dead in what was believed to be a case of suicide. Toxicological screening was performed to assist in establishing the cause of death.
Blood alcohol was determined using GC-FID (Thermo Scientific). When screening for organic xenobiotics, preparation consisted of liquid-liquid extraction of 1 mL of blood collected with sodium fluoride preservative at both acid and alkaline pH by ternary solvent (dichloromethane/hexane/ethyl acetate, 50/40/10, v/v/v). After evaporation, the dry residue was reconstituted in mobile phase at initial condition. The first half of the sample was directly analysed by LC-MS and DAD (Agilent 1100-MSD). Separation was performed on a Macherey-Nagel Nucleodur C18 Gravity column (150 mm x 2 mm, 3 µm) with a mobile phase gradient at 0.5 mL/min (methanol / water 5 mM ammonium formate with 0.2% formic acid). For the second part, after evaporation to dryness and acetylation, analysis was performed on full-scan mode with a GC-MS (Thermo Scientific) equipped with a Varian CP-Sil 8 CB Low Bleed/MS column (25 m × 0.25 mm × 0.25 µm). All substances found were subsequently quantified by LC-MS after addition of internal standard.
In both cases, high blood concentration of citalopram was found (5.54 and 0.93 mg/L respectively) related to its main metabolite desmethylcitalopram (0.19 and 0.17 mg/L respectively). Citalopram was associated with various other psychoactive substances. In the first case, a toxic level of alimemazine (0.77 mg/L) was discovered as well as a therapeutic level of zolpidem (0.16 mg/L), nordazepam (0.53 mg/L) and cyamemazine (0.35 mg/L with 0.86 mg/L of norcyamemazine). In the second case, a high blood alcohol level was also found (3.53 ± 0.17 g/L) alongside various analgesics within therapeutic ranges (dextropropoxyphene, paracetamol and tramadol at 0.27, 3.7 and 0.6 mg/L respectively).
Considering both cases, citalopram was identified within the toxic range (over 0.5 mg/L). Numerous CYP enzymes are involved in citalopram metabolism especially CYP2D6, CYP3A4 and CYP2C19. Use of citalopram with CYP2C19 inhibitors is not recommended by the FDA. Based on this recommendation, no significant pharmacokinetic drug interactions were found among citalopram and the others drugs reported. In those cases, the metabolizer status was not investigated. The overdose of citalopram appears to be responsible for the death, even though presence of other drugs may have potentiated its toxic effects.

P263. TOXICOLOGY-AIDED POSTMORTEM FORENSIC HUMAN IDENTIFICATION: A CASE REPORT

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Forensic toxicology is not commonly considered in cases where forensic human identification is needed. In cases of medico-legal interest, fingerprints, dental impressions, visual identification and DNA normally move forensic human identification forward.
This case report aims to illustrate how forensic toxicology may function as aide in forensic human identification cases in which rapid tentative identification is needed in order to move a medico-legal death investigation forward but when such identification by traditional routes is either not possible or delayed.
A case of dismembered human remains left in a suitcase at a central city location is described. The dismembered body had no head, arms or hands making identification by traditional means difficult. CCTV footage allowed police to identify a possible suspect who was initially apprehended but had to be released from custody just a day after his arrest due to lack of evidence. Witness interviews suggested the suspect's missing roommate as the possible victim and the family of the missing person provided DNA exemplars for forensic human identification use as well as prescription history that included methadone, escitalopram and mirtazapine. Forensic toxicology screens were undertaken in an ABFT-accredited laboratory in biological fluids (central blood and urine) collected at autopsy from the various human body parts using standard forensic analytical procedures (including ethanol and related volatiles by headspace GC-FID, and drug screens by ELISA and full-scan GC-MS).
The analytical results obtained by the forensic toxicology laboratory matched the medical prescriptions provided

by the family of the missing person. Specifically, in both blood and urine methadone, citalopram/escitalopam and mirtazapine were detected. The urine was also found to contain EDDP, cocaine, norcocaine, AME, EME, levamisole, lidocaine and its metabolite, 6-monoacetylmorphine, morphine, codeine and nicotine/cotinine. The toxicology screening results which were completed several days before DNA evidence conclusively matched the body parts to the family of the missing person, were presented to the forensic pathologist and investigator working on this case allowing them to proceed with their medico-legal investigations in a timely fashion based on the tentative forensic human identification match achieved via forensic toxicology.
The case report demonstrates how forensic toxicology may aid forensic human identification in cases of medico-legal interest. Traditional identification methods could not be immediately applied to this case as the remains were discovered with no head, teeth or fingers. DNA, although eventually successful, lagged in time in a case were time was of the essence. The information provided by the forensic toxicologists expedited the medico-legal death investigation. It is important for forensic toxicologists and other forensic practitioners to "think outside the box" and to realise the potential power of forensic toxicology including in cases of forensic human identification.

P264. POST-MORTEM TOXICOLOGY IN DECEASED PREVIOUSLY ARRESTED FOR DRUGRELATED OFFENCES

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Multiple arrests for use of illicit drugs and/or impaired driving are strong indicators of a personality disorder and substance abuse problems. Furthermore, studies show that mortality rates are considerable higher among people who use illicit drugs.
This study investigated the demographic profiles, the cause and manner of death and the concentrations of drugs identified in autopsy blood from people with multiple arrests for illicit drug use and/or driving under the influence of drugs (DUID).
A forensic toxicology database (TOXBASE) was used to compile information about the age and gender of the deceased, the cause (poisoning or other) and manner of death (accident, suicide or undetermined) of 3943 people with multiple drug offences.
Over a period of 18 years 1807 (46%) repeat offenders died from drug overdose (poisoning) compared with 2136 (54%) deaths by other causes. The death was poisoning (overdose) in 46% of multiple offenders compared with only 14% without any previous arrests. Male gender was more prevalent in repeat offenders (89%) compared with all forensic autopsies (74% males). The poisoning death was accidental in 54% of victims with multiple previous arrests. Poly-drug use was common and four or more substances were identified in 44% of poisoning deaths compared with 18% of non-poisoning deaths. The top-five psychoactive drugs were ethanol, morphine (from heroin), diazepam, amphetamines, and THC. The median concentrations of these drugs were not much different between poisoning and non-poisoning causes of death in repeat offenders.
Death from poisoning (overdose) was more common in people with several previous arrests for illicit drug use and/or DUID. Early intervention and treatment for substance abuse might have helped to prevent a premature intoxication death.

P265. DIAMORPHINE-RELATED-FATALITIES IN JEDDAH, SAUDI ARABIA

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The fatalities-involving heroin whose autopsies were performed and tested in the Medico-Legal Center and Poison Control and Forensic Medical Chemistry Center, Jeddah, Saudi Arabia were investigated in order to evaluate the relationship between the mode and cause of death and the laboratory autopsy findings using an LC-ESI-MS-MS. The method was developed for the determination of opioids and their metabolites in unhydrolysed post-mortem specimens. Data was collected for the investigation of heroin related fatalities was between January 2011 and December 2014.
The aim of this study was to report the Blood, vitreous humor, urine and tissues levels of heroin metabolites: 6-Monoacetylmorphine (6-MAM), 6-Acetylcodeine (AC), Morphine (MOR), codeine and Morphine Glucueronides in deaths involving heroin or morphine and to report the range of concentrations detected in real cases to understand their contribution to heroin intoxication. Saudi Arabia weather is very hot most of the year, therefore, the stability of 6-MA and 6-AC was investigated.
Thirty positive post-mortem cases were involved in the current study. The death in 27 cases was attributed solely to heroin intoxication. The majority of heroin poisoning in our autopsy population 27 cases (90%) was attributed to accidental mode of death; the mode of death was not identified in 2 cases (7%); about 43% (13 cases) of these cases were tested positive for other CNS substances. One death was attributed to morphine sulfate related fatalities. The heroin marker (6-MAM) was detected in in 27 cases. Blood, vitreous humor and urine samples were available in most of these case and 6-MAM levels were in the range 0.005-4.87, 18-243 and not detected to 2.778 µg/mL with average and median concentrations of 0.021, 0.100, 0.477 and 6, 91, 130 µg/mL, respectively; morphine levels were in the range 0.005-4.87, 3-891, not detected-50.401 µg/mL with average and median concentrations of 0.391, 0.173, 5.371 and 0.121, 0.91, 2.590 µg/mL, respectively.
Although the weather in Saudi Arabia is very hot most of the year which made the 6-MAM hydrolysis to morphine more likely to happened in most cases, the addition of sodium fluoride as a preservative save 6-MAM from converting to morphine in most of the cases. Additionally, the availability of urine and vitreous humor sample provided

valuable information of which opioids has been administrated. The immunoassay results especially the non-specific reagents for blood and vitreous humor were found not accurate and not recommended to be used in the case of lacking urine samples. In the current work heroin metabolites have been detected in biological fluid and tissue i.e. liver, kidney, gastric content, bile, liver, stomach and brain; new information on the distribution of these analytes were reported; AC a heroin biomarker, for example, was detected in gastric content for the first time in the current work.

P266. RELATIONSHIP BETWEEN ETHANOL, CARBON MONOXIDE AND METAHEMOGLOBIN IN BLOOD SAMPLES IN FORENSIC CASES.

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Many reports have been published about the role of carbon monoxide (CO), actually Carboxyhemoglobin (COHb) and Ethanol (EtOH) in fire fatalities, but possible physiological interaction between these toxic agents is still under discussion. Furthermore, Methemoglobin (MetHb) is not widely considered in fire episodes. MetHb is an oxidized form of Hemoglobin (Hb), which could be generated by redox imbalance induced by the exposure to several chemical agents. The simultaneous relationship of MetHb and other substances with death is under debate.
The aim of the present study was to investigate the combined effect of EtOH, COHb and MetHb poisoning in forensic cases (n=45) from different judicial regions and to establish the relationship of these three parameters in determining the cause of death.
Blood samples from 45 victims were obtained and tested for: a) EtOH by Head-Space Gas Chromatography with FID detector (HS-GC/FID), b) COHb and c) MetHB. For these latter determinations a co-oximeter was used. A Systematic Toxicological Analysis was also performed in order to detect toxic organic compounds, psychotropic and drugs of abuse, using Liquid-Liquid extraction or Solid-Phase extraction. GC-MS was employed to identify these compounds. Toxic compounds routinely measured (methanol, aldehydes and other volatile compounds), gave negative results on the 45 cases. Neither drugs of abuse, nor psychotropic were detected. According to their COHb levels cases were divided into 4 groups: COHb: 1 – 12 % (n=10), 12 – 30 % (n=14), 30 - 50 % (n=13) and > 50 % (n=8). EtOH levels were analyzed according to intoxication criterions: lower 2 g/L (n=35) or higher 2 g/L (n=10). Considering a beginning of intoxication with values above 10%, the MetHb results obtained were divided in: cases with MetHb <10% (n = 11) and cases with MetHb > 10 (n = 34). The results were analyzed linking the three parameters. Statistical analysis showed that %COHb and %MetHb blood concentration were not independent variables with χ^2: 10.87 (theoretic χ^2: 2.09, df: 9, α: 0.05). A relationship between COHb/MetHb > 1 was indicate of a possible predominance of CO poisoning by incomplete combustion of carbon compounds. A relationship COHb/MetHb < 1 was indicate of an intoxication by combustion of compounds which mainly release nitrogen oxides, which cause the oxidation of Hb to MetHb. We found no correlation between EtOH and COHb, and no correlation between EtOH and MetHb. Tridimensional graphics, COHb/MetHb vs ETOH, allowed us to infer the type of poisoning predominating in each group.
The proposed relationship will help to infer the prevailing type of intoxication in forensic cases, and the atmosphere in which the person was at the time of his death.

P267. DETERMINATION OF 3-MMC AND IDENTIFICATION OF ITS METABOLITES BY GC-EI-MS-MS AND GC-EI/PCI-MS IN POST-MORTEM BIOLOGICAL MATERIAL

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Recently, more than 50 cathinone derivatives have been identified in Europe [1]. The EU early warning system receives on the average one notification of a new substance per week. Some of such agents have become preferred products on the market of illegal psychoactive substances, the best example being mephedrone (4-MMC). Nevertheless, in recent years, the predominating factor has been the appearance of new synthetic cathinone derivatives, among them 3-MMC, being an uncontrolled spatial isomer of mephedrone.
The objective of the present study was: 1) to develop and validate a method for determining blood 3-MMC by GC-EI-MS-MS; 2) to identify 3-MMC metabolites in post-mortem biological material by GC-EI/PCI-MS prior to and following enzymatic digestion.
Blood and urine samples secured in the course of autopsies prior to and following digestion with β-glucuronidase with arylsulphatase were subjected to solid phase extraction (SPE) using C18-RP columns manufactured by Agilent. Prior to the analysis, the extracts were derivatized employing a mixture of acetic anhydride and pyridine (3:2, v/v). Determinations of 3-MMC using the internal standard of mephedron-d3 were done by the GC-EI-MS-MS method in the MRM mode. Identification of the metabolites in post-mortem material was performed using the GC-EI/PCI-MS method in the full scan of mass spectra mode.
The employment of GC-EI-MS-MS and GC-EI/PCI-MS allowed for: 1) determination of 3-MMC (4400 ng/ml) in a blood sample using the internal mephedron-d3 standard; 2) identification of its metabolites nor-3-MMC, dihydro-3-MMC, nor-dihydro-3-MMC, hydroxytolyl-3-MMC, nor-hydroxytolyl-3-MMC, 3-carboxy-3-MMC, 3-carboxy-dihydro-3-MMC. Detailed data on pharmacology and toxicology of synthetic cathinones are limited. Identification studies of the metabolism of new psychoactive substances are of significance in achieving better understanding of the toxicity of such compounds (e.g. the effect of genetic alterations in the metabolism, the xenobiotic-xenobiotic interaction).

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P268. **DEVELOPMENT OF A FAST METHOD USING GC/MS FOR THE DETECTION OF 41 PHARMACEUTICALS AND ILLICIT DRUGS IN BLOOD FOLLOWING LIQUID-LIQUID EXTRACTION**

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A Toxicology Lab faces increasing numbers of cases (autopsies or clinical) most of which require fast delivery of trustworthy results. These needs, especially in clinical cases, lead to the necessity for a fast, screening method for a large number of target molecules in biological samples.
The aim of the present study was the development of a fast screening method using Gas Chromatography – Mass Spectrometry (GC-MS) for the detection of 41 pharmaceuticals and illicit drugs in blood samples, after liquid-liquid extraction without the evaporation step of the solvent.
In sample pretreatment step, alkaline liquid-liquid extraction (pH=12) was used, without the evaporation step of the solvent.Analysis was performed on a GC (Agilent Technologies 7890A) combined with an Mass Spectrometer (Agilent Technologies 5975C inrtXL EI/CI MSD with Triple-Axis Detector). The proposed screening method was applied to a total duration of 20 min and utilized a Optima-5-ms, 30 m x 250 μm x 0.25 μm (Scitech Scientific) column with the following temperature program: initial 120oC and Increasing to 300oC (15oC/min). Injection of 1 ul of sample was done through a split-splitless injector operating at 300 oC.
Representative compounds included propofol, diazepam, olanzapine, citalopram, phenobarbital, papaverine, biperiden in total of 41 drugs were selected for the optimization experiments. In sample pretreatment step, alkaline liquid-liquid extraction (pH=12) was used. Three different non-toxic and relative low volatility solvents were tested (butyl acetate, ethyl acetate and 1-octanol). Butyl acetate was chosen as the extracting solvent for the subsequent experiments. Diverse blood sample / solvent ratios (v/v) (4:1, 2:1, 1:1 and 2:5) were used to find a compromise optimum ratio. The final compromise optimal conditions (by comparing the peak areas) were achived by using: 1ml sample (blood), 500 μl (pH=12) of potassium carbonate and 250 μl of butyl acetate.Using the developed screening method a large number of drugs could be detected: anesthetics, benzodiazepines, antipsychotics, antiepileptics, opiates, cocaine, cannabinoids and amphetamines. To enhance the identification potential, avoid false identifications (false positives) and increase productivity Deconvolution Reporting Software (DRS) for GC/MS was optimized and applied for data analysis. DRS offers functionalities that automatically combine analytical results from a selected spectral library. We also applied a new analytical utility namely, Retention Time Lock Database Library thus achieving very reproducible retention times and further improving analyte identification.
The developed screening method was applied for the initial screening of ninety (90) samples (clinical and forensic) at the Laboratory of Forensic Medicine and Toxicology of the University of Thessaloniki and the Laboratory of Forensic Service of Ministry of Justice of Thessaloniki. In many clinical cases, this procedure succeeded to give a fast result in severe poisoning cases indicating directly the cause of the intoxication. Now the method is under validation for each drug, so that in addition to qualitative results to have and the relevant quantitative.

P269. **ANALYSIS OF BUTANE AND PROPANE IN POST-MORTEM BIOLOGICAL SAMPLES BY GC-MS**

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Inhalants' abuse in order to "get high" has become increasingly popular among young people and represents a social problem in many countries worldwide, especially because the wide availability on the market of gases having psychoactive properties. The intake methods of inhalants include inhaling directly from a container by "sniffing" or "snorting", inhaling ("huffing") the substance from a soaked rag, or breathing the fumes from a plastic bag ("bagging"). Death or severe morbidity due to the intake of these substances may be accidental or intentional. Students, adolescents and/or young-adults are the most frequently involved and a wide list of possible sources of propellants is available including common objects such as anti-perspiration aerosol deodorant, glue, shoe polish, gasoline, lighter fluids, and spray paint. In Italy, one of the most common forms of inhalant abuse consists in sniffing gas directly from camping gas refill cylinders, which is a widespread phenomenon among the prison population that is allowed to use camping gas stoves by law. Despite the prevalence and potential risks related to the abuse of these substances, very little is reported in literature about both the exact physiopathological mechanisms of butane/propane intoxication and the best analytical procedures to identify these substances in biological samples. Authors want to give their contribution in setting a valiant procedure for the analysis of butane and propane in post-mortem biological samples. Starting from a lethal case occurred in a prison, where a 29-year-old prisoner was found dead by his cellmate, we describe the sampling approach and the analytical processes that led us to the identification of volatile alkanes in the biological samples collected during the autopsy.
Biological samples were collected during the autopsy and immediately stored in gas-tight glass vials, crimped with teflon coated septa. An Agilent 6890N GC was used, combined with a headspace gas autosampler, and equipped with an Agilent Select Permanent Gases column, made of two capillary columns set in parallel: a molecular sieve 5 Å PLOT capillary column and a Porabond Q. The temperature programme was as follows: 100°C, held for 2 min, and raised at 10°C/min to 250°C; the injector (splitless mode) set to 100°C and the interface MS temperature to 230°C. The detection was performed with an Agilent 5973 mass spectrometer, operating in the electron ionization

mode at 70 eV.
The toxicological analyses performed on the biological samples collected during the autopsy showed high concentrations of propane and butane in all the collected fluids and specimens. Quantitative results from peripheral blood, heart blood, vitreous humour, liver, lung, heart, brain / cerebral cortex, fat tissue, kidney have permitted to evaluate the magnitude of the intoxication. Furthermore the propane and butane identified in the air samples collected from the bronchus and from emphysematous bubbles confirmed the volatile alkanes exposure.
The sampling approach adopted by forensic pathologists and forensic toxicologists and the analyses by GC-MS play a key role to obtain the forensic identification of butane and propane in biological samples and meaningful quantitative data.

P270. DETERMINATION OF PARAQUAT IN BIOLOGICAL SPECIMENS OF THE HOMICIDAL POISONING CASE EXHUMED AFTER 23 MONTHS

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Paraquat is a bis-quaternary ammonium compound that has been widely used as nonselective herbicide. Paraquat is rapidly absorbed and taken up most avidly by lung, kidney, liver, and muscle tissue. Blood paraquat levels decline rapidly, whereas paraquat levels of various organs obtained at autopsy show remarkable accumulation. Ingestion of as little as 2-4 g, or 10-20 mL, of concentrated 20 % paraquat solution has resulted in death without appropriate emergency measures. After ingestion of paraquat there is pain and swelling in the mouth and throat, and oral ulcerations may be visible. Acute intoxications are also characterized by abdominal and substantial chest pain, renal failure, pulmonary fibrosis, coma, and death. Because of toxicity, currently the production, storage, sales, and use of paraquat were prohibited by law. We present on the death by poisoning of an 82-year-old woman related to a series of murders. Exhumation of the corpse was done 23 months after burial and revealed she had been intoxicated with paraquat. The suspect, daughter-in-law, confessed later having mixed a little rice powder coated with paraquat in her food for disguise food stuff. To receive a payout of their insurance, she killed ex-husband and present husband in the same way, and even inflicted an injury on her daughter by the same toxic effect of paraquat as a lung disease
The aims were to determine the disposition of paraquat in various specimens of a decomposed body for forensic toxicology and to prevent the incidence of similar crimes.
Complexity of matrix due to decomposition and low concentration of biological specimen due to long term exposure to a low level and hospitalization before dying were considered in method development. To maximize extraction efficiency, samples were sonnicated with more than twice the amount of ethanol for 1 hr. and concentrated completely. The reconstituted samples by water were performed by solid phase extraction (SPE) cartridge with weak cation exchange (WCX). Paraquat was analysed by ultra phase liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) in positive ionisation mode on a HILIC analytical column (1.7 micron, 2.1×100 mm) and the injection volume was 5 µL. A gradient elution of acetonitrile and water of 0.1M ammonium formate with 0.1% formic acid was used as a mobile phase. The UPLC-MS/MS system coupled with an electrospray ionization (ESI) source was performed in multiple reaction monitoring (MRM) mode. The transitions of paraquat executed as following : m/z 93→171 for paraquat, m/z 529→112 for rocuronium using as an internal standard.
There was no evidence as the cause of death in the forensic autopsy, and just only she displayed pathological alteration of the lungs to the medical opinion during hospitalization. As a result of toxicological testing in autopsy specimens, paraquat was found in her stomach, liver, spleen, kidney, lung tissue and the pelvic bone at concentrations of 1.5, 1.3, 5.5, 4.2, 0.8 and 2.3 ng/g, respectively, but not detected to whole blood and body fluid.
These results suggest that the deceased was exposed to paraquat several days before she died, and show the disposition of paraquat in various specimens of a decomposed body for forensic toxicology. Blood and body fluid were inadaptable for determination of paraquat in such intoxication cases. In addition to, these results will be helpful to prevent similar crimes.

P271. LEVAMISOLE ADULTERATED COCAINE AND PULMONARY VASCULITIS

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Levamisole, an anthelmintic for veterinary use, is widely used as adulterant for cocaine since early 2000 in the United States and Europe. The reasons for this practice may be explained by it partial conversion in aminorex, an amphetamine-like drug. This latter acts synergically with cocaine inducing a longer effect. On the other hand, aminorex is proven to cause pulmonary hypertension (IPH).
The aims are: the description of a new case of isolated pulmonary vasculitis due to levamisole-adulterated cocaine; to review the recent literature on human pharmacokinetics of levamisole and aminorex; to propose an explanation of the uncommon appearance of levaminsole/amiorex-pure pulmonary complications
Analysis by gas chromatography-mass spectrometry of acid, neutral and alkali was performed on tissues from a 51-years-old man who died suddenly while being taken to hospital because of bizarre behavior.
Severe pulmonary edema and lymphocytic cuffing around small pulmonary vessels was identified during the autopsy. Heart showed microscopic evidence of the typical remodeling changes associated with chronic stimulant abuse.

The cocaine concentration was 740 ng/mL while the benzoylecgonine concentration was 1800 ng/mL. Notable also was the presence of cocaethylene and levamisole, although these were not quantitated.
The population pharmacokinetics of levamisole and aminorex have been characterized. Levamisole can be detected for up to 36 hours after ingestion in serum, but concentrations of aminorex in serum never exceeded the LOQ. Nevertheless, the rate of conversion of levamisole to aminorex, or the amount of aminorex and duration of exposure to levamisole that is required to cause IPH, have not yet been determined.
Although death in this case was almost certainly the result of profound heart disease, the presence of pulmonary vasculitis in the lungs is clear. Hence, when levamisole is detected, lung examination may be helpful to study the evolution of vasculitis diseases. Observations suggested that pulmonary vasculitis due to levamisole/aminorex may convert into hemorrhagic vasculitis in a long time.

P272. DETERMINATION OF CONTAMINATED SUBSTANCE IN HERBAL MEDICATED SPIRIT IN BANGKOK BY GAS CHROMATOGRAPHY WITH FLAME IONIZATION DETECTOR

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Herbal medicated spirits are herbs submerged in an ethanol solution for a period of time to extract curative properties. It is a commonly found form of herbal medicine in Thailand. However, its curative properties and adverse side effects are yet to be scientifically determined. The production of herbal medicated spirit is mostly done in the local household without any quality testing and we believe that this could lead to contamination of unwanted substances.
The aim of the study is to determine the contaminating agents in herbal medicated spirit sold in Bangkok metropolitan area by using gas chromatography with flame ionization detector (GC/FID) and gas chromatography with mass spectrometry (GC/MS).
17 herbal medicated spirits were bought from markets in Bangkok. Physical properties of the samples were recorded (Color, Odor and pH). All samples were performed with GC/FID and GC/MS. One hundred microliters of each herbal medicated spirit was mixed with 100 µL of n-butanol (1 mg/mL) as the internal standard and was transferred to a 22 mL headspace vial. The vials were crimped sealed and placed on the instrument for analysis.
Determination with GC/MS revealed multiple contaminants (methanol, n-propanol, benzene, and ethyl acetate) through matching with MS library. Gas chromatography with flame ionization demonstrated methanol in all samples.
Samples of herbal medicated spirit sold within the Bangkok metropolitan area are contaminated with methanol which might be added to lower the production cost of the herbal medicated spirit by increasing the volume. However, the toxic nature of methanol could lead to severe adverse effect among consumers.

P273 EVALUATION OF THE DNA DAMAGING POTENTIAL OF ANTIDOTE OXIME K048 IN A549 AND HACAT CELL LINES

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Pyridinium oximes are used as successful antidotes against poisoning by various organophosphate compounds, mainly due to their ability to protect unphosphorylated acetylcholinesterase (AChE) and/or reactivate phosphorylated AChE. Previous studies with the K048 oxime (N-[4-(4-hydroxyiminomethylpyridinio) butyl]-4-carbamoyl-pyridinium dibromide), a representative of a new generation of pyridinium oximes, have suggested its acceptable cytotoxicity and low potential for infliction of primary DNA damage in human peripheral blood lymphocytes in vitro and rat blood cells in vivo.
In the present study, we evaluated the genotoxic potency of K048 oxime on the human lung adenocarcinoma epithelial cell line A549 and human keratinocyte cell line HaCaT in vitro. Since both cell lines are known for non-neuromuscular AChE expression, this study also aimed to clarify the mutual relationships between DNA damage and AChE activity.
Both cell lines were cultivated in RPMI growth medium supplemented with glutamine, heat-inactivated fetal bovine serum, and antibiotics. Prior to the treatment, cells were seeded in 6-well plates (3x105 cells/mL). The K048 oxime was tested in the concentration range 0.0073 to 2 mM, which was well below the IC50 value, established previously. After 30 minutes of treatment, aliquots of cells were used for the preparation of agarose microgels according to standard protocol for the alkaline comet assay. Slides were analyzed under fluorescent microscope using the Comet Assay IV analysis system (Perceptive Instruments Ltd., UK). The level of DNA damage was evaluated based on comet tail length, tail intensity and tail moment.
Our results show that both cell lines expressed a low level of spontaneous DNA damage. The short exposure time used here was obviously not critical for the induction of severe DNA lesions. The most prominent finding was the concentration-dependent decrease of genotoxicity, which was observed in both cell lines. Primary DNA damage was slightly increased as compared to the negative control only at two lowest concentrations tested. This observation points to a potential antioxidative efficacy of the tested compound, which has to be elucidated in future investigations.
Taken together, our results confirm high biocompatibility for the K048 oxime, which has already been recognized in

previous studies and also speaks in favour of further studies on this compound as a promising antidote for human use.

P274 DEFECTIVE LIQUID NITROGEN TANKS AND DEATH DUE TO INHALATION OF NITROGEN IN A CONFINED ENVIRONMENT: TOXICOLOGICAL ISSUES ABOUT THE CAUSE OF DEATH

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Nitrogen is physiologically an inert, nontoxic gas, not involved in human metabolism and it is considered to be a simple asphyxiate gas. Displacing the oxygen, it causes a decrease in the environmental air and the availability in trade respirators. The decrease of the atmospheric oxygen to values of 15-16% leads to a rapid unconsciousness and death within minutes. In these cases the assessment of nitrogen concentrations in human fluids and tissues is complicated by the high percentage of the gas in the atmosphere (78%). This presentation discusses the results of the chemical- toxicological performed for the diagnosis of cause of death.
A 37-year old male, veterinarian expert in artificial insemination of cattle, was found unresponsive, sitting in the driving seat of his car with the head resting on the steering wheel. Every resuscitation effort was unsuccessful. He was lastly heard alive on the phone just 15 minutes before. Inside the trunk of the car, three liquid nitrogen tanks, that he used to carry for working purposes, were found; two of them were defective with clear losses because of incomplete lock.
For toxicological investigations, in glass vials previously closed with Teflon plugs and saturated with helium, were sampled cardiac blood, the vitreous humor and the pericardial fluid. The blood was collected by intracardiac injection, before the beginning of the autopsy. Samples of femoral blood, urine, bile and tissues (liver, lungs, brain, kidney, adipose tissues) were placed in the same type of vials, not saturated with helium.
With the spectrophotometric technique were determined the hemoglobin and its most important derivatives, obtaining the following results: HbTot 14,1 g/dL, HbO2 27,3%, RHb 67,4%, HbCO 4,2%, HbMet 0,8%. The nitrogen distribution in liquids and tissues was performed by GC/MS technique, with sampling of the headspace. At the same time were evaluated other respiratory gases, O2 and CO2. For the gas chromatographic separation was used a dedicated column (GS-GASPRO – J&W113-4332). The following masses were monitored: O2 32 m/z; N2 28 m/z, CO2 44 m/z . Their percentage composition in the gaseous mixture injected into the GC/MS, was calculated by internal normalization. The percentages of nitrogen were greater than the atmospheric pressure in the cardiac blood (85,23%), in the blood of the right lung (80,73%), in femoral blood (81,01%) and adipose tissue (80,28%).
The case presented is related to an event occurring during profession and it happened accidentally. The evaluation of the distribution of respiratory gases in the autopsy samples and the determination of hemoglobin derivatives completed the diagnostic picture on the causes of death (nitrogen asphyxia), which otherwise would have to rely only on investigations of the scene and the circumstances surrounding the death.

P275 VALIDATION OF A NEW AND FAST METHOD FOR THE MAIN MOLECULAR SPECIE OF THE PHOSPHATIDYL ETHANOL (PETH 16:0-18:1) MEASUREMENT IN BLOOD BV LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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Phosphatidyl Ethanol (PEth) is a "pathological" phospolipid, formed via the action of phospholipase D only in the presence of ethanol. the blood PEth concentration has been demonstrated to correlate with the amount of alcohol consumed, even though the relationship varies considerably between individuals. Main molecular specie is PEth 16:0-18:1. At the moment it is the most promising marker of alcohol abuse. Purpose of the paper is the valuation of a fast method for PEth 16:0-18:1 measurement in whole blood by HPLC-MS/MS. A comparison with the routine method proposed by Helander will be done. The pre-analytical pahse consists in a precipitation of the proteins in a single step starting from 100 µL of whole blood, deuterated internal standard d5-PEth 16:0-18:1 included. Calibration is possible by a certificated standard produced by Red- Hot Diagnostics (Sweden). The analytical phase is performed on HPLC-MS/MS (Nexera Shimatzu and 4000QTRAP ABSciex) with ESI source in negative ionization. Chromatographic seprataion is obtained by a C18 columns with a binary gradient. run time is 5 minutes.
Imprecision: two samples of whole blood at 0.3 to 0.6 M (concentrations which correspond to the cut-off and doube the cut-off) were analyzed in 5 replicates in 3 different analytical series (intra assay imprecision) with CV% respectively 4.2% and 2.9% and in 5 different analytical series (inter assays imprecision) with CV% 6% and 4.3 respectively. Linearity: calculated by dilution scale pf the calibrator: between 0.078 and 5.0 M. LOQ: determined as signla/noise ratio greater than 10. In matrix it is 0.1 M. Recovery: two samples in water and two samples in whole blood at the same concentration of 1 M were prepared. When we use the following formula (100 x Area ratio average blood sample/area ratio average water samples), recovery percentage was 99.6%. Comparison with the Helander routin method: $Y = 1.02 X + 0.04$; $R^2=0.9899$.
This fast method has all the requirements to be useful in a routine laboratory with an elevated number of PEth tests.

social program

53rd TIAFT meeting 2015
FIRENZE
August 30th - September 4th, 2015

Social program

Sunday, August 30th

OPENING CEREMONY

Salone dei "Cinquecento"

5.00pm **Opening Ceremony of the 53rd Annual Meeting of TIAFT**
Hee-Sun Chung, TIAFT President

5.20pm **Greetings from the Authorities**

6.00pm **Greetings from the President of the 53rd Annual Meeting of TIAFT**
Elisabetta Bertol

6.15pm **Opening Lecture by Marilyn Huestis:**
"A conversation with Lucrezia Borgia: Saint or Sinner?"

Social program

Sunday, August 30th

Welcome Reception

Salone dei Cinquecento

7.00pm Renaissance Show in Piazza della Signoria

8.00pm Welcome Cocktail in Palazzo Borghese

Social program

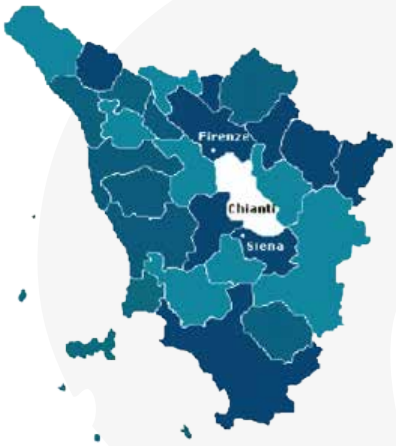
Wednesday, September 2nd

Half-Day Excursion

Chianti Hills

1.00pm Departure to the Chianti Hills
The excursion includes a visit of a prestigious and ultra-modern cellar (Antinori-Bargino), as well as smaller and ancient traditional wineries, with wine tasting, and other local sites.

7.00pm Country dinner in Badia a Passignano in the shade of cypress trees and medieval walls.



Social program

Friday, September 4th

Social Dinner and Awards Presentation

Salone dei Cinquecento

8.30pm **Social Dinner and Awards Presentation**

Social Dinner Entertainment

An unforgettable surprise with an original musical production

The 53rd TIAFT meeting supports the production of Re-Edith, a show for music and video-art, developed around the French singer Edith Piaf. Re-Edith will be presented in the magnificent Salone dei 500, heart of the Italian Renaissance, during the social dinner on September 4th 2015. This initiative was born as a mission to support modern Italian art, in its multiple forms and expressions, where music and modern performance art meet.The idea for the project arises from the reading of a collection of unpublished letters "Mon Amour bleu" that Edith Piaf wrote between 1951 and 1952 to a secret lover, but they were discovered only in 2009. The lyrics for the songs in the show are the result of an accurate and delicate process of partial cancellation of the words of each letter. The aim was to compose a new text, through a work of poetic synthesis as a unique unit without interruptions. Absence and subtraction: these are the two elements through which her character has been explored, providing a description that overcomes the public persona that everyone knows. Her words have been distilled and deprived of their daily lives and time references, in order to make them part of a universal time-space dimension, part of a poetry that belongs to everyone and that everyone understands and shares.The entirely original music has been created for a "solo" voice, an acoustic session made of four cellos, and an electronic session. The projection of video images, which are also original, is the visual expression of what the music represents.

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TIAFT 2015 welcomes the following exhibitors

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SRA INSTRUMENTS
TECAN ITALIA
UCT

Meeting Venue

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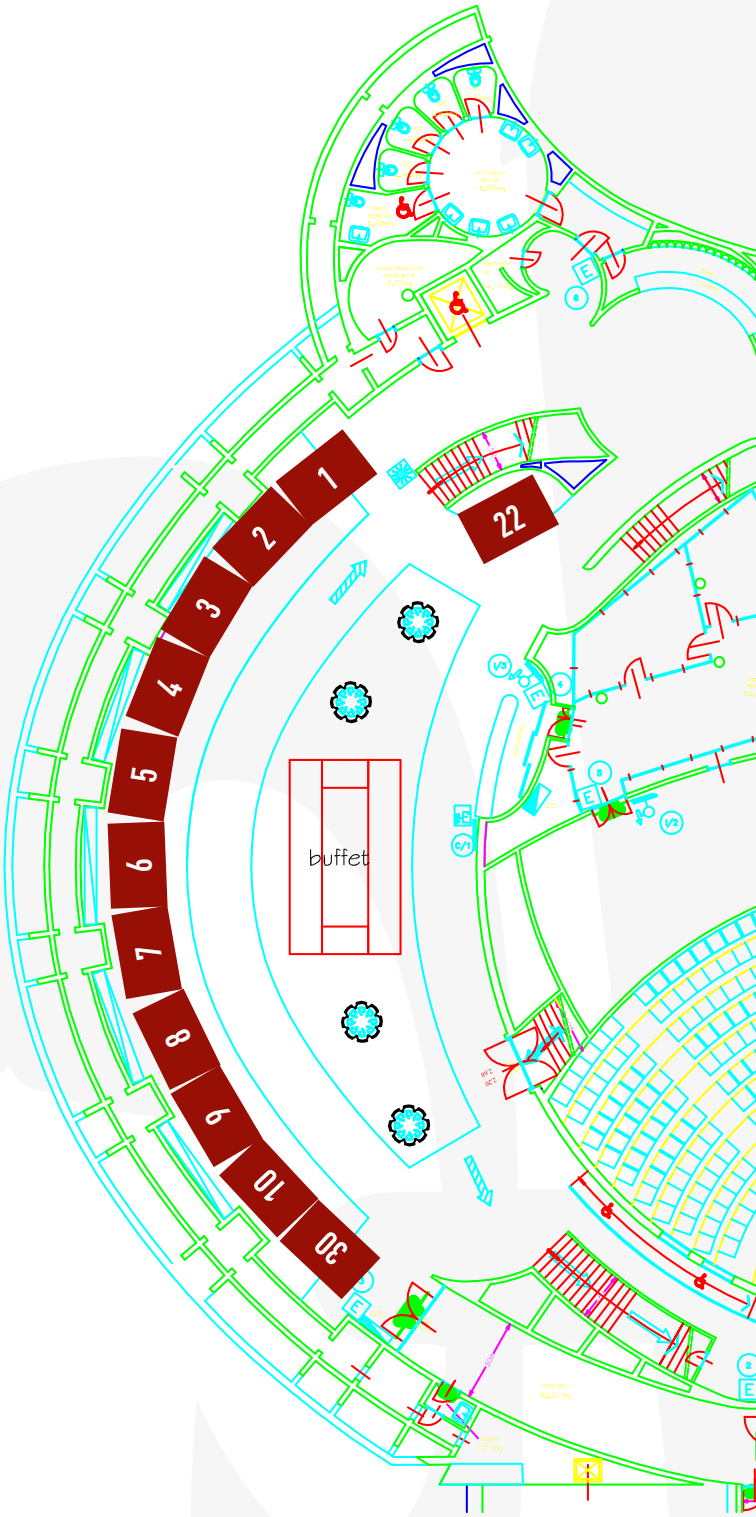
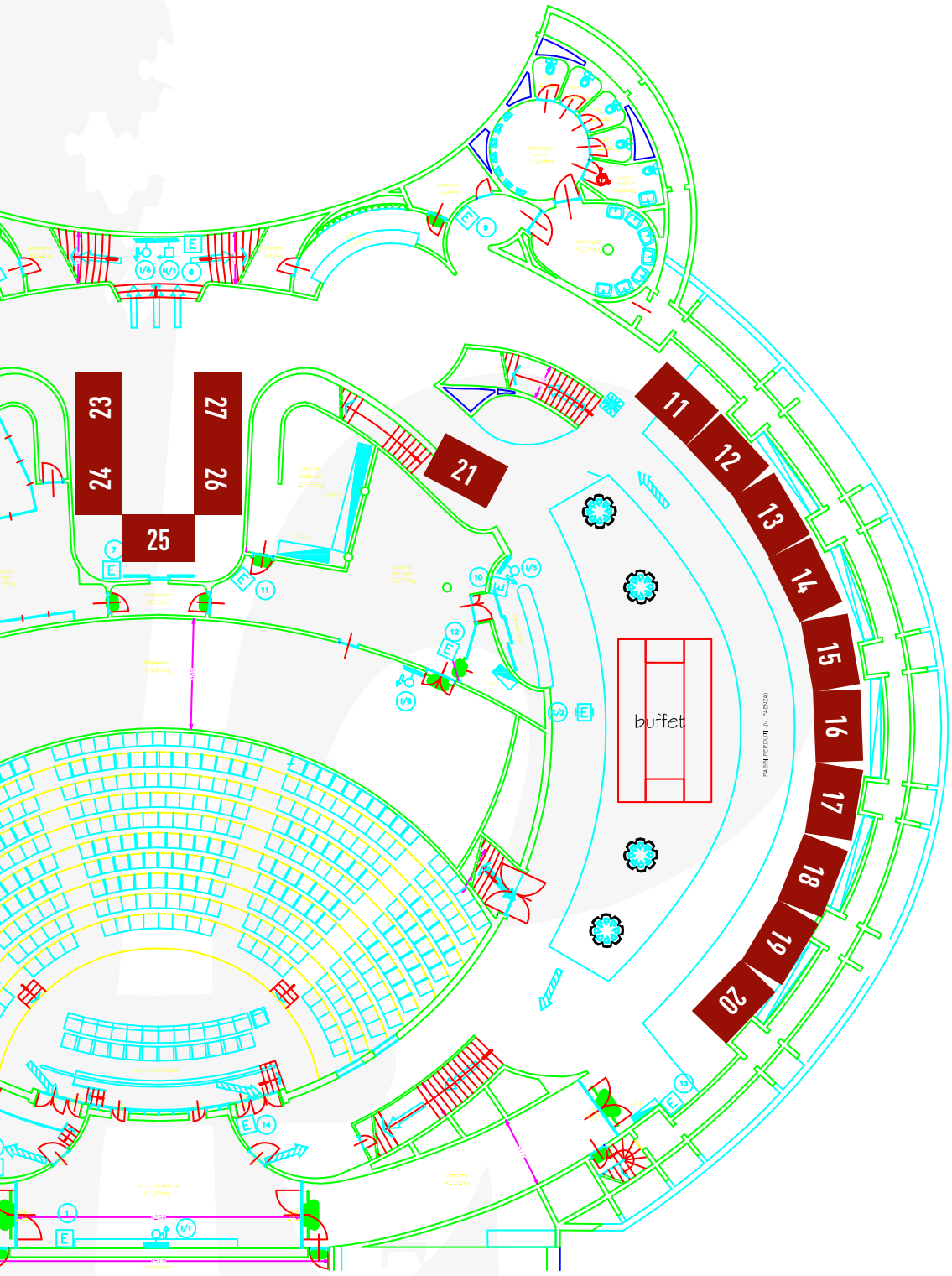
Conference Center Floor Plan

STAND	COMPANY NAME
01	BSN
02	RANDOX TOXICOLOGY
03-04	AGILENT TECHNOLOGIES
05-06	THERMO FISHER SCIENTIFIC
07-08	WATERS
09	SIGMA-ALDRICH
10	CERILLIANT CORP.
11	BIOTAGE
12	BRUKER DALTONIK
13	CHIRON
14	GREINER BIO-ONE
15	NEOGEN
16	AB SCIEX
17	LGC STANDARDS
18	SECURETEC DETEKTIONS-SYSTEME
19	TECAN
20	UCT
21	DORNER
22	ACQ Science
23	NAL VON MINDEN
24	GERSTEL
25	TIAFT Meeting 2016
26	PHYTRONIX TECH.
27	LIPOMED
30	ARK Diagnostic

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buffet

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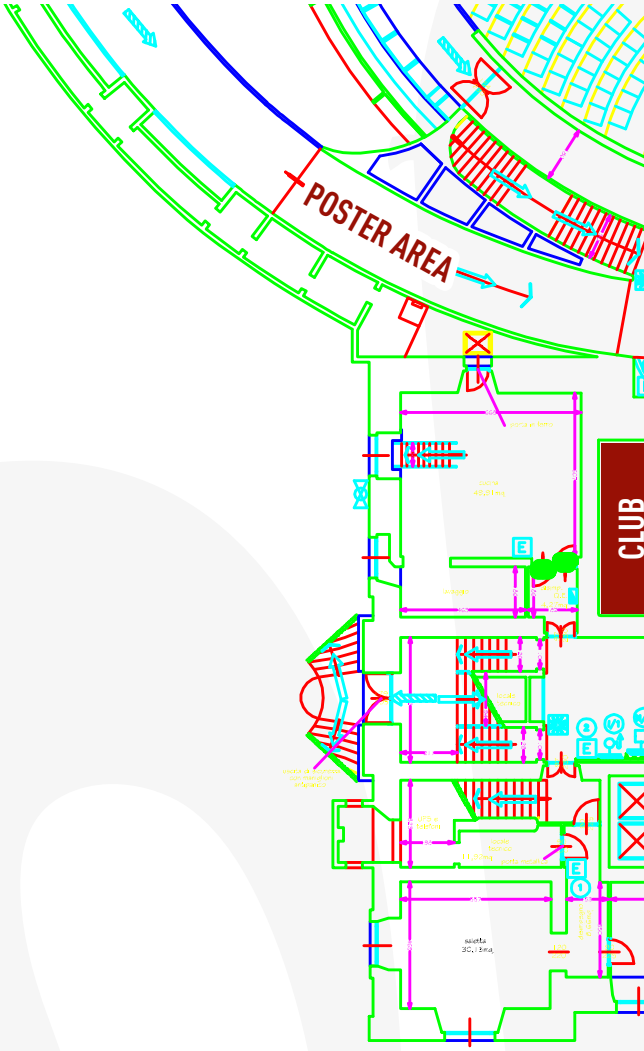
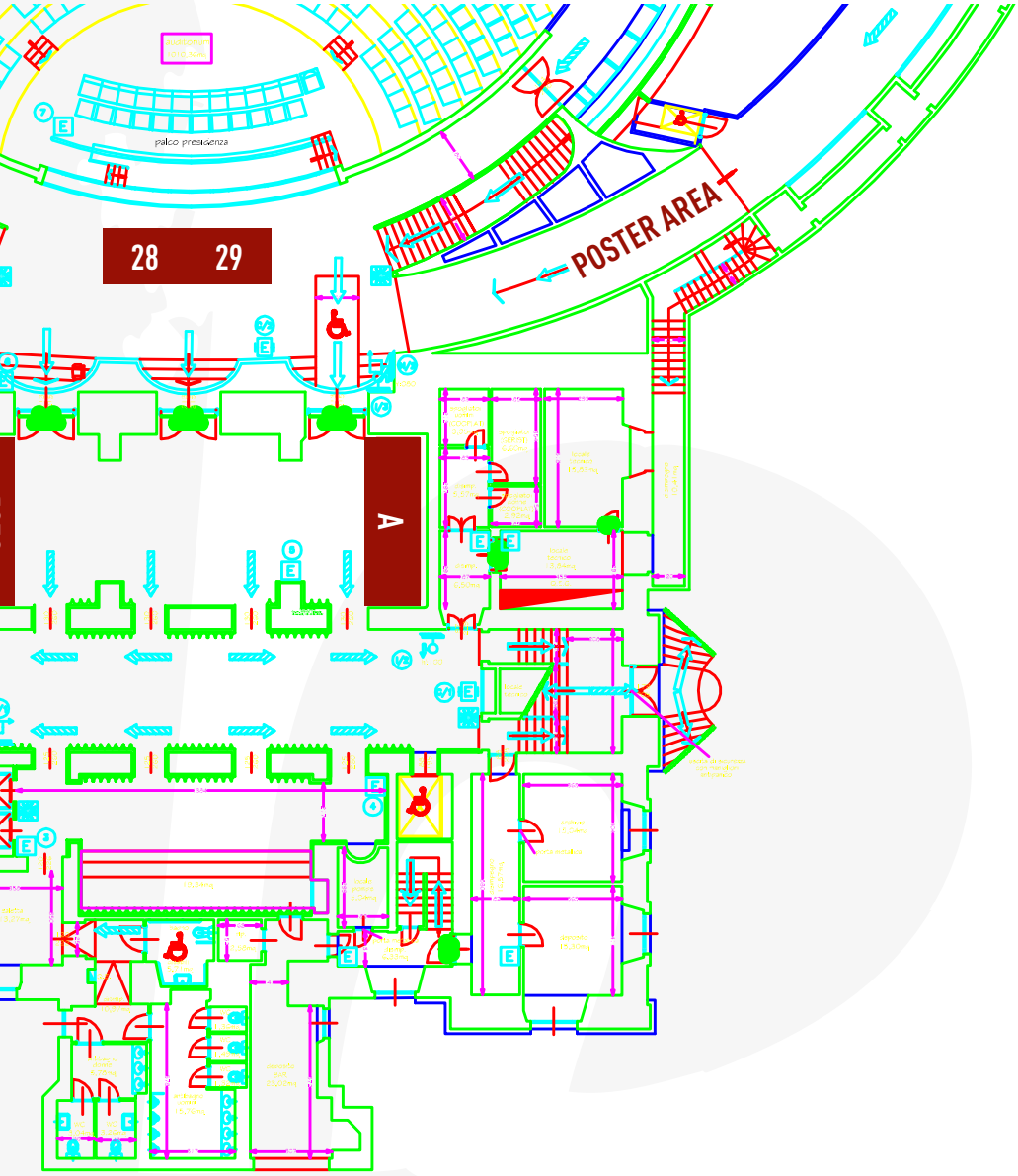
Exhibitors Floor Plan and Poster Area

STAND	COMPANY NAME
28-29	SIEMENS
CLUB	SHIMADZU
A	TIAFT Meeting 2019 & TIAFT SECRETARIAT

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29
POSTER AREA
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General information

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General information

CONGRESS VENUE

Palazzo dei Congressi - Firenze Fiera
Piazza Adua, 1, Firenze FI

The TIAFT Meeting will be held at the Palazzo dei Congressi; this venue will successfully promote and boost the city of Firenze as a high-level conference and exhibition location.
Palazzo dei Congressi (conference centre), hosted in a 19th century villa built by the Strozzi family, today it's perfectly restored and houses antique furniture. Located inside the 18th century Villa Vittoria, Palazzo dei Congressi boasts a prestigious auditorium which can host up to 1,000 people. There is also an exhibiting area of 920 sq m, along with other halls which can host 50, 80 (Sala Onice -Onyx Hall) and 200 people (Sala Verde - Green Hall) respectively, and which are positioned within the 4 floors of the villa. Surrounded by a centuries-old garden, Palazzo dei Congressi and its adjacent Lemon House is completely accessible on foot, in the heart of the city.

ORGANIZING SECRETARIAT

PLS Educational
Via della Mattonaia, 17
50121 Florence (Italy)
Phone: +39 055 24621
E-mail: tiaft@promoleader.com
Website: www.tiaft2015.com

SECRETARIAT ONSITE OPENING HOURS

August 30th, 2015	8:00am - 4:00pm
August 31st, 2015	8:00am - 6:30pm
September 1st 2015	8:00am - 6:30pm
September 2nd 2015	8:00am - 2:00pm
September 3rd 2015	8:00am - 6:30pm
September 4th 2015	8:00am - 6:30pm

General information

REGISTRATIONS
On-site payment can be made in cash (EURO) or credit card (only VISA and Mastercard).

Registration fee includes:
- full access to the scientific sessions and exhibition
- congress bag with the final program and abstract book
- access to the Opening Ceremony and Welcome Reception (upon availability)
- half-day excursion and Country Dinner (upon availability)
- lunches (August 31, September 1-2-3-4)
- refreshment breaks
- certificate of attendance
- name badge
- TIAFT 2015 Meeting t-shirt

Accompanying person registration fee includes:
- access to the Opening Ceremony and Welcome Reception (upon availability)
- half-day excursion and Country Dinner (upon availability)
- lunches (August 31, September 1-2-3-4)
- refreshment breaks
- name badge
- TIAFT 2015 Meeting t-shirt

TIAFT 2015 CONGRESS CARD
Please download your TIAFT 2015 CONGRESS CARD at www.conventionbureau.it (it provides special offers and discounted fees at museums, historical sites, shops, restaurants, wellness centers, golf clubs, bike rental stands, car rental agencies, sightseeing tours, taxis, and airport transfer with private driver).

OFFICIAL LANGUAGE
The official language of the Congress is English. No simultaneous translation will be available.

WIFI
There is a free access to wireless internet in all aparts of the congress venue. Select the "**TIAFT2015**" network name and enter the access code "**tiaft2015**"

INTERNATIONAL PHONE CALLS
For all your calls abroad Italy, dial 00 followed by the country code 39.

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LIABILITY AND INSURANCE

The Organizing Secretariat and Committee accept no liability for personal injuries or loss or damage of property belonging to the congress delegates, either during or as a result of the congress or during any of the social events. It is recommended that participants arrange their own personal health, accident and travel insurance.

ORAL PRESENTATIONS

Oral presentation time is 10 minutes, plus 3 minutes for discussion.
All submitted and accepted oral presentations have been subjected to peer review and can be found in the program.

POSTERS

The Poster session will be held in "Ballatoi area" and will be divided into two groups, one from Monday to Tuesday and one from Thursday to Friday; posters change will be done on Wednesday morning from each poster author.
Poster dimensions should not exceed 70 cm width x 100 cm height.
All submitted and accepted poster abstracts have been subjected to peer review and can be found in the program.

SLIDE CENTER

Presentations must be delivered to the Slide Center room at least one hour before the session starts; oral presentation speakers are kindly requested to upload the presentation with a USB device.
Personal computers in the session rooms will not be accepted.
Slide Center opening hours will follow the Organizing Secretariat opening hours.

USEFUL TELEPHONE NUMBERS

Taxi Service: 0039 055 4390 - 0039 055 4242
Medical Emergency: 118
Other Emergencies: 112

CLIMATE AND CLOTHING

The weather in Florence in August and September is usually very hot and sunny with daily temperatures ranging from 25°C to 35°C.

CURRENCY

EUR (€) is the official currency in Italy. Money can be changed at the main train stations, international airports, major banks, exchenge bureau, most large hotels and the post office. All major credit cards are accepted in most hotels, restaurants and shops.

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