IMaSS

Italian Mass Spectrometry Society

Forensic Mass Spectrometry

Chairman FMS Verona 2016
Prof. Franco Tagliaro

Director of the Unit of Forensic Medicine,
Dept. of Diagnostics and Public Health,
University of Verona, Verona, Italy

Co - Chairman FMS Verona 2016
Dr. Giacomo Musile

Post-Doc Researcher at the Unit of Forensic Medicine,
Dept. of Diagnostics and Public Health,
University of Verona, Verona, Italy

November 11th, 2016
Aula Magna Gavazzi
Policlinico G.B. Rossi,
via Bengasi, 4 - 37134
Verona, Italy

Local Organising Committee

Giacomo Musile (University of Verona, Verona)

Nadia Porpiglia (University of Verona, Verona)

Elena Giacomazzi (University of Verona, Verona)

Organising Committee

Enrico Davoli (Mario Negri Foundation, Milano)

Pietro Franceschi (Edmund Mach Foundation, San Michele all'Adige, Trento)

Ornella Curcuruto (Aptuit, Verona)

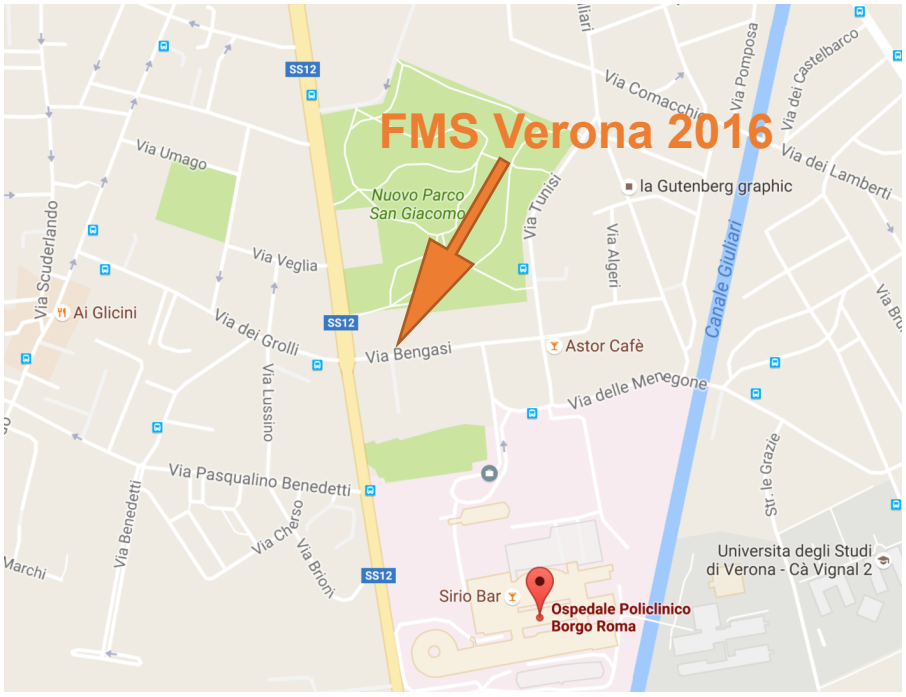
Marco Roverso (CNR, Padova)

Claudio Medana (University of Torino, Torino)

Giacomo Musile (University of Verona, Verona)

Marynka Ulaszewska (Edmund Mach Foundation, San Michele all'Adige, Trento)

Mappa



Forensic Mass Spectrometry

IMaSS
Italian Mass Spectrometry Society

GOLD SPONSORS

SCIEX

BRUKER

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

SHIMADZU
Excellence in Science

ISOTOPIC RATIO® INSTITUTE

ThermoFisher SCIENTIFIC

SILVER SPONSORS

LGC

phenomenex®
. . . breaking with tradition℠

Scientific Program - FMS Verona 2016 (November 11th, 2016)

REGISTRATION OF PARTICIPANTS 9:00 - 9:30

09:30 - 09:50	**OPENING ADDRESS FMS VERONA 2016**
09:50 - 10:20	**Chemical criminal profiling using Matrix assisted Laser desorption Mass Spectrometry Imaging.** *Simona Francese* *Reader in Bioscience and Chemistry, Sheffield Hallam University, United Kingdom.*
10:20 - 10:40	**Explosives and mass spectrometric chemical analysis.** *Francesco Saverio Romolo* *Unit of Legal Medicine, Department SAIMLAL, Sapienza University of Rome, Italy.*
10:40 - 11:00	**Forensic toxicology: a journey towards the unknown.** *Claudio De Nardi* *Thermo Fisher Scientific GmbH, Germany.*
11:00 - 11:30	**Coffee break - Poster Session**
11:30 - 11:50	**Mass spectrometry applications in forensic entomology.** *Stefano Vanin* *School of Applied Sciences, University of Huddersfield, United Kingdom.*
11:50 - 12:10	**Evaluation of time dependent protein signatures in dried blood spots.** *Thalassa S.E. Valkenburg [1], Garry L. Corthals [2]* *[1] University of Leicester, INTREPID Forensics, United Kingdom.* *[2] University of Amsterdam, Van 't Hoff Institute for Molecular Sciences, Netherlands.*
12:10 - 12:30	**Mass spectrometry applications in polymeric trace evidence characterisation.** *Valerio Causin* *Department of Chemical Science, University of Padova, Italy.*
12:30 - 13:30	**Lunch time**
13:30 - 13:50	**Fast and comprehensive LC-MS^n identification for drugs and drugs of abuse in clinical research and forensics.** *Elisa Basso [1], Anthony Drury [2]* *[1] Bruker Daltonics S.r.l., Macerata, Italy.* *[2] Bruker UK Ltd., Coventry, United Kingdom*
13:50 - 14:10	**Cannabinoids analysis in keratin matrix: development of an innovative method by TURBOFLOW™ HPLC-MS/MS for quantitative analysis.** *Manuela Fontana [1], Serena Fanara [1], Sergio Indelicato [2], Valentina Castelli [1], Enza Billone [1], Francesca Di Gaudio [1,3].* *[1] Mass Spectrometry Laboratory for Clinical Risk and Quality Control (CQRC), A.O.U.P. "P. Giaccone", University of Palermo, Italy.* *[2] Thermo Fisher Scientific, Les Ulis, France.* *[3] Department of Pathobiology and Medical Forensic Biotechnology (DiBiMEF), A.O.U.P. "P. Giaccone", University of Palermo, Italy.*

14:10 - 14:30	**Current status of non-targeted liquid chromatography-tandem mass spectrometry in forensic toxicology.** *Herbert Oberacher* *Institute of Legal Medicine and Core Facility Metabolomics, Medical University of Innsbruck, Austria.*
14:30 - 14:50	**New approaches for target and untarget screening in Toxicology with LC-MS/MS SWATHTM analysis.** *Samuele Scurati* *Sciex Italy, Milano, Italy.*
14:50 - 15:20	**Coffee break - Poster Session**
15:20 - 15:40	**Systematic Toxicological Screening Using LC-MS.** *Rob Lee, Michelle Wood, Simone Donzelli* *Waters Corporation, Waters Italia, Italy.*
15:40 - 16:00	**New challenges for electron ionization-mass spectrometry.** *Pierangela Palma. Veronica Termopoli, Giorgio Famiglini, Maurizio Piergiovanni, Achille Cappiello* *Dept of Pure and Applied Sciences, LC-MS Group, University of Urbino, Italy.*
16:00 - 16:20	**CE-MS applied in forensic and clinical toxicology.** *Rossella Gottardo* *Department of Diagnostics and Public Health, Section of Forensic Medicine, University of Verona, Italy.*
16:20 - 16:40	**Application of mass spectrometry to forensic sciences: GC-MS, LC-MS and MALDI Imaging.** *Veronica Mainini. Davide Giovanni Vecchietti, Giuseppe Scollo* *Shimadzu Italia Srl, Italy*
16:40 - 17:00	**A novel screening method for 69 new psychoactive substances and 5 amphetamines in blood by LC-MS/MS and application to real cases.** *Fabio Vaiano. Valeria Catalani, Diego Palumbo, Elisabetta Bertol* *Department of Health Science, University of Florence, Italy.*
17:00 - 17:20	**Elucidation of elemental composition of new psychoactive substances by isotopic fine structure analysis using high resolution/high accuracy Orbitrap mass spectrometry.** *Giampietro Frison. Flavio Zancanaro, Luca Zamengo, Gianpaola Tedeschi, Samuela Frasson, Luca Gino Sbrogiò* *Laboratory of Environmental Hygiene and Forensic Toxicology, Department of Prevention, Azienda ULSS 12 Veneziana, Mestre, Italy.*
17:20 - 17:40	**MALDI-TOF mass spectrometry for the rapid identification of peptide and protein doping agents in seized materials.** *Marco Roverso [1], Donata Favretto [2] and Roberta Seraglia [1]* *[1] CNR-ICMATE, Padova, Italy.* *[2] Unit of Legal Medicine and Toxicology, Dept SCTV, University of Padova, Italy.*
17:40 - 18:00	**Closing Remarks**

List of Oral Contributions

List of Vendor Seminars

List of Poster Contributions

4 Soprintendenza per i Beni Archeologici del Lazio micaela.angle@beniculturali.it
5 Museo Nazionale Preistorico Etnografico "Luigi Pigorini" claudio.cavazzuti@gmail.com

List of Oral Contributions

Mass spectrometry applications in polymeric trace evidence characterisation.

Valerio Causin

Department of Chemical Science, University of PadovaUniversità di Padova, via Marzolo 1, 35131 Padova, Italy.

In his daily casework, a forensic scientist confronts himself with traces, which are the remnants of an activity and as such they contain the most basic 'material or physical' information on a crime.
The ubiquity of polymeric materials in everyday life make polymeric traces items very probably found on a crime scene. The most common include textile fibres that are shed during a struggle, paint chips transferred or reflectors broken in a car accident, or adhesive tape that is often used in kidnappings, bomb manufacturing, robberies or concealing of dead bodies. However, the array of possible polymeric materials that a forensic scientist could be called to deal with is very vast, and goes from latex gloves, to bits of polyurethane foam, from resins contained in inks to lubricants in condoms.
Polymers are peculiars chemicals and much information is contained within their complexity and heterogeneity [1]. Synthesis and manufacturing processes modify the material on the molecular scale, tuning its microstructure, solid state structure, morphology and physical-mechanical properties.
Practically no plastic object is made of 100% polymer. Most polymers in fact do not possess adequate aesthetical and/or functional properties to fit common end uses, so additives are extensively used in industrial practice.
Among the many analytical techniques in the forensic toolbox, mass spectrometry is probably one of the most underutilised in the characterisation of polymeric items. In this communication, the strengths and the limitations of mass spectrometry in this field will be discussed, with a particular focus on the elucidation of the formulation of the item and of the molecular weight of its matrix.

References
[1] Causin V., Polymers on the Crime Scene – Forensic Analysis of Polymeric Trace Evidence. Springer: New York (2015).

Cannabinoids analysis in keratin marix: development of an innovative method by TURBOFLOW™ HPLC-MS/MS for quantitative analysis.

Manuela Fontana [1], Serena Fanara [1], Sergio Indelicato [2], Valentina Castelli [1], Enza Billone [1], Francesca Di Gaudio [1,3].

[1] Mass Spectrometry Laboratory for Clinical Risk and Quality Control (CQRC), A.O.U.P. "P. Giaccone", University of Palermo, Palermo, Italy.
[2] Thermo Fisher Scientific, Les Ulis, France.
[3] Department of Pathobiology and Medical Forensic Biotechnology (DiBiMEF), A.O.U.P. "P. Giaccone", University of Palermo, Palermo, Italy.

Cannabis derivatives are even today the most commonly used illicit drugs in Italy. The cut-off values, proposed in Italy by the GTFI Guidelines, are very low, especially for the keratinic matrix, and, because of it, they are needed sensitive and specific methods which can confirm the consumption of cannabis.
The aim of the present work was the development of an advanced analytical method, based on LC-MS/MS, for the identification and determination in hair of Δ9-Tetrahydrocannabinol (THC) together with its major metabolite 11-nor-9-Carboxy-Δ9-Tetrahydrocannabinol (THC-COOH).
The sample pre-treatment procedure is based on a basic hydrolysis followed by a LLE step. Analytical phase was performed using the Thermo Scientific™ Transcend™ II System which gives an online sample clean-up by TurboFlow™ technology prior to HPLC separation and MS/MS analysis.
During method development, they have been performed some different experimental tests, regarding the analytical conditions. The adequate selectivity of TurboFlow™ in the sample cleaning, and the possibility to inject more volumes of sample than the normal LC-MS techniques, they mean an increased sensitivity.
According to the aim of the work, some steps of sample preparation, such as LLE, they were replaced with a simple hydrolyzate filtration and with a direct injection into TurboFlow™ system.
The method developed by TurboFlow™-HPLC-MS/MS, without the LLE step, allows an accurate quantification of cannabinoids in the hair. This technique, showing an adequate sensitivity, can be compared and preferred to the routine techniques used. The rapid preparation of the sample generates a considerable saving of time and materials, and a reduction of possible errors caused by the operator.

Chemical criminal profiling using Matrix assisted Laser desorption Mass Spectrometry Imaging.

Simona Francese

Reader in Bioscience and Chemistry, Sheffield Hallam University, United Kingdom.

Since 2008, the Fingermark Research Group at Sheffield Hallam University has been developing novel Matrix Assisted Laser Desorption Ionisation Mass Spectrometry profiling and imaging methodologies to recover additional intelligence from fingermarks. Lifestyle, personal and activity information prior to accidental mark deposition, have given rise to a "new type of criminal profiling" based no longer on behavioural science but on chemistry. This intelligence potentially narrows the pool of suspects, provides investigative leads and informs judicial debates and crucial collaborations, mainly with the Home Office' CAST and West Yorkshire Police have been established. The technology is now being trialled on crime scene marks, with the view of integrating it into forensic fingermark examination workflows. Various tandem analytical workflows have been developed, showing that additional physical and chemical information (such as illicit drugs, blood and other excreted substances) can be retrieved following initial fingermark development, thus indicating operational feasibility of the proposed methodologies.

Elucidation of elemental composition of new psychoactive substances by isotopic fine structure analysis using high resolution/high accuracy Orbitrap mass spectrometry.

Giampietro Frison, Flavio Zancanaro, Luca Zamengo, Gianpaola Tedeschi, Samuela Frasson, Luca Gino Sbrogiò

Laboratory of Environmental Hygiene and Forensic Toxicology, Department of Prevention, Azienda ULSS 12 Veneziana, P.le Giustiniani 11e/2, I-30174 Mestre (Venezia), Italy.

The impressive market growth of new psychoactive substances (NPS) is a complex phenomenon that affects both the health and security of citizens worldwide. Amphetamine-related designer drugs as well as synthetic cannabinoids represent the NPS that most frequently appear for some years now on the recreational drug market.
Clinical and forensic toxicology laboratories are challenged every day by the analytical aspects of the NPS phenomenon. They are required to identify NPS, sometimes very quickly and often without the availability of reference standards or analytical data from scientific literature.
Liquid chromatography coupled with high resolution/high accuracy Orbitrap mass spectrometry (LC-HRMS), particularly when operating at 100.000 resolving power, may be very helpful in elucidating elemental composition and structural characteristics of NPS.
The application of LC-HRMS allows the accurate mass measurement of MH^+ ionic species and the study of MH^+ collision-induced product ions obtained in MS/MS experiments. In addition, the comparison of experimental and calculated MH^+ isotopic patterns, and above all the accurate examination of the isotopic fine structure (IFS) of the M+1, M+2, M+3 isotopic peaks (which may be very complex because of the contribution of ^{13}C, ^{2}H, ^{15}N, ^{18}O isotopes) relative to the monoisotopic M+0 peak, strongly support and often confirm the assignment of elemental formulae to the NPS analyzed.
In the last few years about forty NPS have been identified in both seized materials and biological fluids at the Author's laboratory using LC-HRMS. Representative analytical findings will be presented and discussed.

References

[1] Frison G. et al, Italian Journal on Addiction, **4**, 57 (2014).
[2] Frison G. et al, Rapid Communications in Mass Spectrometry, **29**, 1196 (2016).
[3] Frison G. et al, Rapid Communications in Mass Spectrometry, **30**, 151 (2016).
[4] Frison G. et al, Forensic Science International, **265**, 144 (2016).

CE-MS applied in forensic and clinical toxicology.

Rossella Gottardo

Department of Diagnostics and Public Health, Section of Forensic Medicine, University of Verona, P.le Scuro, 10- 37134 Verona, Italy.

Capillary electrophoresis (CE) and particularly its hyphenation with mass spectrometry (CE-MS) has so far gained only moderate attention in forensic toxicology and in other forensic sciences. However, CE-MS could become an important analytical tool in the hands of forensic analysts, as it combines the high efficiency and resolution of CE with the selectivity and sensitivity of MS. Moreover, CE deals with minute amounts of sample and buffers and therefore looks more suitable than HPLC for interfacing with electrospray ionisation (ESI), typically affected by ion suppression phenomena.
After several years of neglect, in recent times, the CE-MS hyphenation has recently started to be used in pharmaceutical analysis and in clinical and forensic toxicology showing great potential in qualitative and quantitative determination of therapeutic and abusive drugs and drug metabolites in body fluids and tissues.
More recently, the improvement of ion sources to meet the specificity of CE and the introduction of High Resolution Mass Spectrometry have extended the potential of CE-MS techniques, opening interesting fields of application in the forensic sciences.
These subjects will be discussed with examples of applications to real forensic cases.

Current status of non-targeted liquid chromatography-tandem mass spectrometry in forensic toxicology.

Herbert Oberacher

Institute of Legal Medicine and Core Facility Metabolomics, Medical University of Innsbruck, Muellerstrasse 44, 6020 Innsbruck, Austria.

A core task of any forensic toxicology lab is providing comprehensive information on the chemical composition of evidence. This mission can only be accomplished by combining efficient detection techniques with reliable identification procedures. A competent approach for the sensitive detection of a large variety of potentially toxic compounds is liquid chromatography/tandem mass spectrometry (LC/MS/MS) [2, 3]. The concept of non-targeted analysis is realized by applying either data-dependent or data-independent acquisition strategies. Non-targeted LC/MS/MS produces informative features for subsequent compound identification. Of particular importance is the availability of fragmentation information. Additionally, retention times and isotopic distributions may be used. During the identification process, extracted features are matched against data sets obtained from reference standards. The reference data is often stored in databases. Although state-of-the-art tandem mass spectral databases enable automated compound identification with high sensitivity and specificity, the final decision on identity is still taken by an expert who carefully reviews the provided arguments.

References

[1] Oberacher, H., Arnhard, K., *Bioanalysis* **7,** 2825 (2015).
[2] Oberacher, H., Arnhard, K., *Trends Anal. Chem.* http://dx.doi.org/10.1016/j.trac.2015.12.019.

New challenges for electron ionization-mass spectrometry.

Pierangela Palma, Veronica Termopoli, Giorgio Famiglini, Maurizio Piergiovanni, Achille Cappiello

Università di Urbino Carlo Bo, Dipartimento di Scienze Pure ed Applicate, LC-MS Group, Piazza Rinascimento 6 – 61029 Urbino, Italy.

In electron ionization (EI) mass spectrometry (MS) the analytes are ionized in the gas-phase by means of high-energy electrons. The high energy involved in the ionization process also promotes the extensive fragmentation of the molecule. Highly reproducible and characteristic spectra of the analytes can be collected and compared against library reference spectra for compound identification. EI is the standard technique used in gas chromatography (GC)-MS. Solvent and sample restrictions cause difficulties in creating a similar EI-based liquid chromatography (LC)-MS interface. Our research group developed an LC-MS interface, called Direct-EI, which combines, in a single instrument, the identification advantages of library searchable EI spectra with the separation power of an LC column, with no matrix effects and no need of a high-resolution instrument [1, 2]. Recently, a new interface design called “Liquid-EI”, LEI, was developed by our group, in which the vaporization of the LC eluate is carried out inside a micro-channel right before entering the ion source [3]. An overview of different applications of forensic interest, involving the three aforementioned techniques, is presented: 1) the determination of targeted and non-targeted compounds in human brain samples [4, 5, 6]; 2) the quali-quantitative assessment of benzodiazepines in drinks [7, 8].

References

[1] P. Palma, G. Famiglini, H. Trufelli, E. Pierini, V. Termopoli, A. Cappiello, *Anal. Bioanal. Chem.* **399**, (8), 2683 (2011).

[2] A. Cappiello, G. Famiglini, P. Palma, E. Pierini, V. Termopoli, H. Trufelli, *Mass Spectrom. Rev.* **30**, 1242 (2011).

[3] A. Cappiello, M. Piergiovanni, G. Famiglini, V. Termopoli, P. Palma, A. Cappiello “DEI vs. LEI: a novel, advanced system for interfacing liquid chromatography and electron ionization mass spectrometry.” Presented at the 64th ASMS Conference on Mass Spectrometry and Allied Topics, San Antonio, TX, USA, June 5-9, 2016.

[4] A. Cappiello, G. Famiglini, P. Palma, V. Termopoli, A. M. Lavezzi, L. Matturri, *Anal. Bioanal. Chem.* **406**, 2779 (2014).

[5] V. Termopoli, G. Famiglini, P. Palma, L. Magrini, A. Cappiello, *Anal. Bioanal. Chem.* **407**, 2463 (2015).

[6] V. Termopoli, G. Famiglini, P. Palma, A. Cappiello “UHPLC-Liquid-EI-MS/MS interface: a new frontier of multiclass environmental risk factors assessment in sudden infant death syndromes.” Presented at the 64th ASMS Conference on Mass Spectrometry and Allied Topics, San Antonio, TX, USA, June 5-9, 2016.

[7] G. Famiglini, F. Capriotti, P. Palma, V. Termopoli, A. Cappiello *J. Anal. Toxicol.* **39** (4), 306-312 (2015).

[8] G. Famiglini, V. Termopoli, P. Palma, A. Cappiello *Electrophoresis* **37** (7-8), 1048 (2016).

Explosives and mass spectrometric chemical analysis.

Francesco Saverio Romolo

Unit of Legal Medicine, Department SAIMLAL, Sapienza University of Rome, Italy Viale Regina Elena 336, 00161 Rome, Italy.

Explosives are chemical substances playing a very important role in many activities such as mining or engineering. Some explosive compounds have other uses such as in medicine (nitrate esters), in agriculture (ammonium nitrate), or in the plastic and paint industry (nitrocellulose), but the word explosive is commonly associated with serious criminal activities. For this reason there is a lot of research about explosives in forensic science. Mass spectrometry is an important tool to obtain analytical information about both bulk and traces explosives. Trace analysis of explosives is very important in bombing-scene investigations to provide valuable information to the investigators at a time when it is most needed and to collect material that is most likely to produce evidence. Trace analysis of explosives is also very important during the examination of places where explosive charges were prepared or vehicles used to transport an improvised explosive device (IED). In both situations mass spectrometry can provide fast and sensitive detection of explosives. Other interesting subjects related to explosives are the analysis of organic gunshot residue and the estimation of the time since discharge of spent cartridges. Analytical information provided by mass spectrometry supported very important criminal investigations worldwide, but without the proper forensic approach, no analytical tool can properly support the security of citizens and the administration of justice[1].

References

[1] F.S. Romolo, G.G. Vadalà, New tecnologies and police activities: security and forensic issues. *Energia, Ambiente e Innovazione* **17**, 22 (2014) (DOI: 10.12910/EAI2014-86). http://www.enea.it/it/produzione-scientifica/pdf-eai/speciale-enea-technologies-for-security/new-technologies.pdf, last access on 15th September 2016.

MALDI-TOF mass spectrometry for the rapid identification of peptide and protein doping agents in seized materials.

Marco Roverso [1], Donata Favretto [2] and Roberta Seraglia [1]

[1] CNR-ICMATE, Padova, Italy.
[2] Medicina Legale e Tossicologia, Dipartimento SCTV, Padova University, Padova, Italy.

Products of pharmaceutical nature and nutritional supplements have received increasing attention by élite and non-professional athletes due to their potential performance enhancing effects. The web and the illicit market both offer a wide range of products, whose origin, properties and safety are not known at all. The risks associated with uncontrolled use of highly potent and/or non-approved pharmaceutical compounds in healthy individuals are of public concern. Materials seized during inspection campaigns need to be tested to assess the magnitude of potential risks on health, and accordingly refer offenders to prosecution. Whereas GC/MS and LC/MS methods are ideal for the investigation of doping substances of low molecular weight, i.e. stimulants and steroids, the analysis of peptides or proteins, typically in the molecular range 1-300 kDa, necessitate different analytical approaches. Matrix-assisted laser desorption/ionization (MALDI)-time-of-flight (TOF)-mass spectrometry (MS), routinely used for the identification of proteins in biological fluids, proved to be a rapid, sensitive and specific tool for the identification of prohibited peptides and proteins in anonymous or (mis)labeled products, syringes, needles or infusion sets.
The sample preparation turned out to be rapid and straightforward. Tiny amounts of powders or liquids can be analyzed without chromatographic separation in less than 30 min. Doping agents belonging to the prohibited class of “Peptide hormones, growth factors and related substances" could be identified in seized materials. Conversely, no active drug was found in fake products labeled with GH claims.

A novel screening method for 69 new psychoactive substances and 5 amphetamines in blood by LC-MS/MS and application to real cases.

Fabio Vaiano, Valeria Catalani, Diego Palumbo, Elisabetta Bertol

[1] Department of Health Science, University of Florence, Italy.

Identification and quantification of new psychoactive substances (NPS), both in biological and non-biological samples, represent a hard challenge for forensic toxicologists. NPS are increasingly emerging on illegal drug market. Many cases of co-consumption of NPS and other substances have also been reported. Hence, the development of analytical methods aiming at the detection of a broad-spectrum of com-pounds (NPS and “traditional” drugs) could be helpful. In this paper, a fully validated screening method in blood for the simultaneous detection of 69 substances, including 64 NPS (28 synthetic cannabinoids,19 synthetic cathinones, 5 phenethylamines, 3 indanes, 2 piperazines, 2 tryptamines, 2 phencyclidine, methoxetamine, ketamine and its metabolite) and 5 amphetamines (amphetamine, methamphetamine, MDMA, MDA, 3,4-methylenedioxy-N-ethylamphetamine − MDEA) by a dynamic multiple reaction monitoring analysis through liquid chromatography − tandem mass spectrometry (LC–MS/MS) is described. This method is very fast, easy to perform and cheap as it only requires the deproteinization of 200 µL of blood sample with acetonitrile. The chromatographic separation is achieved with a C18 column. The analysis is very sensitive, with limits of quantification ranging from 0.1 to 0.5 ng/mL. The method is linear from 1 to 100 ng/mL and the coefficient of determination (R2) was always above 0.9900. Precision and accuracy were acceptable at any quality control level and recovery efficiency range was 72–110%. Matrix effects did not negatively affect the analytical sensitivity.
This method was successfully applied to various real cases.

Evaluation of time dependent protein signatures in dried blood spots.

Thalassa S.E. Valkenburg [1], Garry L. Corthals [2]

[1] University of Leicester, INTREPID Forensics, 154 Upper New Walk, Leicester LE1 1QA, United Kingdom.
[2] University of Amsterdam, Van 't Hoff Institute for Molecular Sciences, Science Park 904, 1090 GS Amsterdam, the Netherlands.

The analysis of blood stains is important in a variety of forensic scenarios. Blood spatter pattern analysis aids in the reconstruction of a crime and DNA is commonly obtained from blood stains to verify a person's identity. The toxicology of blood is rarely tested at crime scenes, but can give the police and the criminal justice system vital clues as to the state of mind of person(s) involved. Finally the use of blood stains to determine the time of deposition related to the time a crime was committed could have an enormous value, but this is not yet widely used. In this research project, liquid chromatography followed by tandem mass spectrometry (LC-MS/MS) is used to study the protein signature of ageing blood spots.
Essentially the method involves the digestion of blood proteins into peptides and subsequent analysis by LC/ESI-MS/MS. Briefly, 10 µl of capillary blood was collected from six volunteers and spotted on human ID bloodstain cards. The blood spots were aged under lab conditions; in the dark with freely circulating air at room temperature. Next, the spots were extracted from the cards followed by a trypsin treatment to digest blood proteins overnight. Sample clean-up was performed using solid phase extraction (SPE) and subsequent reversed phase – high performance liquid chromatography (RP-HPLC) on in-house packed 75 µm columns. Tandem MS was performed in positive ion mode on 2+, 3+ and 4+ ions in the range from 400 to 1250 a.m.u. on a Sciex Triple TOF 5600+. Proteins were identified and will be quantified using standard proteomics software tools.
Up to 185 proteins and 2087 peptide signatures have been identified in the ageing DBS with 1% False Discovery Rate (FDR). A slight increase in the number of different proteins and peptides can be observed from spots sampled at day 0 until samples aged up to 1 week.
The results show interesting differences in the molecular composition of proteins in blood between various time points and beckons further analysis under different conditions; not limited to DBS on human ID bloodstain cards. Once a selection of time dependent markers is established, a similar approach may be used as previously shown by Borchers et al. who detected and quantified 97 proteins in a targeted manner using multiple reaction monitoring (MRM) [1]. This would reduce the method from discovery mode to validation mode, opening the door for high throughput screening and ultimately validating some of the findings from the discovery work.

References

[1] Chambers, A.G., Percy, A.J., Yang, J. and Borchers, C.H. (2015). Multiple Reaction Monitoring Enables Precise Quantification of 97 Proteins in Dried Blood Spots. *Molecular & Cellular Proteomics,* 14(11): 3094-3104.

Mass spectrometry applications in Forensic Entomology.

Stefano Vanin

School of Applied Sciences, University of Huddersfield, Queensgate HD1 3DH Hudderfsield, United Kingdom.

Forensic entomology is the branch of forensic sciences in which insects are used as evidence in legal investigations related to humans or wildlife. The examination, identification and analysis of the insects associated with human remains, combined with the knowledge of insect biology and the meteorological parameters, especially the temperature, can provide a further level of detail in addition to medical and anthropological data in the reconstruction of the events occurred close to the death. In particular, necrophagous insects are useful for the Post-Mortem Interval (PMI) estimation, the post-mortem transfer discovery and for the detection of drugs or poisons if soft tissues are no longer available. In order to answer these questions analytical analyses based also on GC/MS showed their potential in three main domains: 1) Species identification of immature developmental stages 2) Age identification of immature stages and 3) Detection of drugs from insects and insect remains collected from the body or from the crime scene. The first two approaches have been applied mainly focusing on cuticular hydrocarbons, whereas the third one give birth to a discipline called “Entomotoxicology”. The aims of this branch of the knowledge is not only the identification, via analytical methods, of the ingested drugs but as well the understanding of the drug effect on insect development. This point is crucial for the estimation of the minimum time since death on drug abusers. The role of the mass spectrometry in forensic entomology has not yet completely investigated and exploited at the maximum of its potential. In future, a higher interdisciplinary collaboration between analytical and forensic scientists is recommended.

List of Vendor Seminars

Fast and comprehensive LC-MSn identification for drugs and drugs of abuse in clinical research and forensics.

Elisa Basso [1], Anthony Drury [2]

[1] Bruker Daltonics S.r.l., via Cluentina 26/R 62100 Macerata, Italy.
[2] Bruker UK Ltd., Banner Lane, Coventry, CV4 9GH, United Kingdom

There is high demand in clinical research and forensic toxicology for comprehensive, specific and transferable technique that overcome the well-known limitations of current GC-MS, LC-UV/DAD and immunoassay solutions. Liquid chromatography-tandem mass spectrometry (LC-MS) combined with library searching is an emerging screening solution for toxicology research.
With this talk we present ToxtyperTM, a robust and automated research based screening solution for the detection and identification of drugs and drugs of abuse in biological specimens. The workflow was tested, with great results, with regard to method – and result- transferability from lab to lab.

Forensic Toxicology: a Journey Towards the Unknown.

Claudio De Nardi

Thermo Fisher Scientific GmbH, Germany.

Mass Spectrometry associated to Liquid Chromatography is playing a daily increasing role in Forensic Toxicology, allowing scientists to take advantage of databases and spectral libraries for a more and more reliable identification and confirmation of compounds in the most diverse matrices. The introduction of Orbitrap and High Resolution Accurate Mass Spectrometry in this field was the keystone towards untargeted and, most of all, unknown identification and confirmation. The need for a fast and reliable software for data processing and interpretation is now becoming as important as the impressive hardware evolution occurred in recent years.

Systematic Toxicological Screening Using LC-MS.

Rob Lee, Michelle Wood, Simone Donzelli

Waters Corporation, Waters Italia, Italy.

Forensic toxicology laboratories require reliable screening techniques that can detect a wide variety of toxicants in highly complex biological matrices, such as ante and postmortem specimens. This approach uses an UPLC coupled with triple quadrupole. Data is acquired in scanning mode using in-source fragmentation and library searching. Libraries are built by Waters using the NIST format and are dedicated to Toxicology. This method is suited for screening of more than 950 drug substances and metabolites. Runtime from sample to sample is only 15 minutes, including column conditioning. Sample deconvolution and library searching are managed by a dedicated software specific for this kind of application.
This solution has been successfully and routinely used in toxicology laboratories worldwide providing a simple and sensitive method for forensic toxicology screening of compounds in various biological matrices (Hair, blood, plasma, urine, oral fluid).

New approaches for target and untarget screening in Toxicology with LC-MS/MS SWATHTM analysis.

Samuele Scurati

Sciex Italy, via Cappuccini 6 Milano, Italy.

Target and untarget screening in toxicology are challenging goals. To be successful, High Resolution Mass Spectrometers with SWATHTM analysis can be used. SWATH analysis allows the registration of all the high resolution MS and MS/MS spectra with no loss of information. As consequence, a real complete fingerprint of the samples can be acquired with the possibility of a full retrospective analysis and compounds quantitation. Then, all the registered MS and MS/MS spectra can be easily processed using dedicated software and databases containing lists of toxic compounds; in case of new synthetized molecules not yet available in database, the molecular formula identification followed by online search and MS/MS interpretation can help in compounds identification. A real case of Target and Untarget screening will be shown using as an example the analysis of internet available tablets. Data acquired with X500R QTOF will be processed in a real time software session and the process used to identify the compounds will be explained.

Applications of mass spectrometry to forensic sciences: GC-MS, LC-MS and MALDI Imaging

Veronica Mainini, Davide Giovanni Vecchietti, Giuseppe Scollo

Shimadzu Italia Srl (Italy)

Forensic toxicological sample measurement can take two paths commonly referred to as targeted and untargeted analysis. Targeted approaches on triple quadrupole platforms are well established with MRM/SRM detection and quantitation for pre-determined lists of compounds. We will show the possibility to approach targeted analysis both by GCMS and LCMSMS.
For untargeted analysis, general unknown screening acquires full scan MS data to trigger MS/MS spectra at a broad range of collision energies above a predefined treshold. We will describe in detail the application of a nominal mass library acquired with certified reference materials to screen and identify forensic toxicology samples in routine clinical laboratories. The method is designed to acquire MRM and scanning data with polarity switching in a single data file: the MRM provides quantitative results while full scan data help confirming identification.
We will also discuss one of the most recent approaches in forensic field : investigation of latent fingermarks by MALDI Imaging mass spectrometry.

List of Poster Contributions

Incidence of new psychoactive substances in drug abuse within various populations and countries: evidences from hair analysis.

Cristina Bozzolino [1,2], Alberto Salomone [2], Enrico Gerace [2], Daniele Di Corcia [2], Marco Vincenti [1,2]

[1] Dipartimento di Chimica, Università degli Studi di Torino, Via P. Giuria 7, 10125 Torino, Italy – cristina.bozzolino@unito.it
[2] Centro Regionale Antidoping e di Tossicologia "A. Bertinaria", Regione Gonzole 10/1, 10043 Orbassano, Torino, Italy

Introduction. In the last few years, hundreds of new psychoactive substances (NPS) have been introduced in the drug market, mostly sold through internet and directly delivered at home. Although this unprecedented sale mode opened a new scenario for drug dissemination, the real diffusion of NPS, their current market penetration, turnover, and characteristic user population features remains questioned, while traditional drugs are still mostly consumed. The determination of NPS in hair samples was proposed as an effective strategy to study the diffusion of NPS among the investigated populations, since their accumulation in the keratin matrix allowed a perspective overview on poly-abuse and unaware intake of NPS. In this presentation, the results from hair analysis from selected populations of various EU countries and USA are discussed.
Methods. Hair samples were analyzed using two fully validated UHPLC-MS/MS methods, previously published, for 31 stimulant, psychedelic, and dissociative drugs, plus 23 synthetic cannabinoids. The following parameters were investigated: selectivity, specificity, linearity range, LOD, LOQs, intra- and inter-assay precision and accuracy, carryover, recovery, and matrix effects. About 680 real hair samples taken in Italy (600) and New York City (80) were analyzed. Comparison with other EU studies is presented.
Results. Both analytical methods proved highly effective for the quantitative determination of 54 NPS occurring over wide concentration ranges. This allowed us to clearly distinguish occasional (often unaware) intake of certain NPS from chronic abuse. A prevalent diffusion of NPS as recreational drugs, often abused in association with other drugs, was proved especially among young consumers, during weekends, music happenings, and festivals. New strategies to gain information on the specific NPS currently trafficked are also used, including drug checking service during recreational events and the administration of a questionnaire to festival participants combined with hair analysis from donors. A survey of volunteers was combined with hair analysis, showing that about four out of ten nightclub/festival-attending young adult ecstasy users tested positive for bath salts and/or other NPS, despite reporting no lifetime use of these substances. Therefore, results suggest that many ecstasy-using nightclub/festival attendees may be unintentionally using bath salts.
Conclusion. Since most routine analyses on body fluids do not include screening procedures for NPS, clear knowledge of the real consumption of these new drugs in the population is prevented. The introduction of hair analysis as a tool for the detection of NPS diffusion appears to be appropriate within a drug policy strategy.

References
Salomone A., Luciano C., Di Corcia D., Gerace E., Vincenti M., *Drug Test. Anal.* **6** (2014).
Salomone A., Gazzilli G., Di Corcia D., Gerace E., Vincenti M., *Anal. Bioanal. Chem.* **408** (2016).
Palamar JJ., Salomone A., Vincenti M., Cleland CM., *Drug Alcohol Depend* **161** (2016).

Detection of anabolic steroids in bodybuilding dietary supplements.

Enza Billone [1], Daria Catalano [1], Serena Fanara [1], Manuela Fontana [1], Francesca Di Gaudio [1,2]

[1]Mass Spectrometry Laboratory for Clinical Risk and Quality Control (CQRC), A.O.U.P. "P. Giaccone", University of Palermo, Palermo, Italy; daria.catalano@unipa.it
[2]Department of Pathobiology and Medical Forensic Biotechnology (DiBiMEF), A.O.U.P. "P. Giaccone", University of Palermo, Palermo, Italy; francesca.digaudiounipa.it

Sports Federations, such as the World Anti-Doping Agency (WADA), consider the use of Anabolic Androgen Steroids (AAS) in sports as doping and prohibit the use of them. Athletes are periodically subjected to doping controls, by the research of the parent drug and/or their metabolites in urine. In this work, two bodybuilding dietary supplements were analyzed by LC-HRMS/MS in order to verify the absence of illegal substances in them, after recent findings of 17-α-trenbolone (epitrenbolone) in doping control urine samples of athletes consuming such supplements. These kind of nutraceuticals are used by athletes as legal and safe alternatives to AAS to improve their physical performances and are sold worldwide on internet without any control by authorities. A liquid chromatographic high-resolution mass spectrometry method was developed, using the Thermo Scientific™ Q Exactive™ Mass Spectrometer coupled to an ESI ion source. The analysis conducted verified the presence of 17-β-trenbolone and trenbolone acetate in one of the two supplements, thus demonstrating the contamination of the tablets with low concentrations of this anabolic steroid. The diastereoisomers may give 17-α-trenbolone as the primary metabolite.

References

[1] Di Gaudio *et al.*, *Analytica J Cell Physiol.* 231(6):1385-91 Effects of Nandrolone Stimulation on Testosterone Biosynthesis in Leydig Cells (2016).
[2] Thevis *et al.*, *Steroids* 74, 315–321 (2009).
[3] Blackwell *et al.*, *Chemosphere* 138, 901–907 (2015).

Characterization of new psychoactive substances and development of a qualitative and quantitative screening method for 1-cyclohexyl-4-(1,2-diphenylethyl)piperazine (MT-45) in the urinary matrix.

Roberta Ciullo [1], Gabriele Vannutelli [2], Roberta Mazzoni [3], Adolfo Gregori [3], Claudio Medana [4].

[1] Corso di Laurea in Chimica Clinica Forense e dello Sport, Dipartimento di Chimica, Università di Torino, via Giuria 7, 10125, Torino; roberta.ciullo@gmail.com;
[2] Dipartimento di Chimica, Sapienza Università di Roma, piazzale Aldo Moro 5, 00185, Roma;
[3] Sezione di Chimica Esplosivi ed Infiammabili, RIS Roma, viale Tor di Quinto 119, 00191, Roma;
[4]Dipartimento di Biotecnologie Molecolari e Scienze per la Salute, Università di Torino, via Giuria 5, 10125, Torino.

The advent of New Psychoactive Substances is a pervasive phenomenon that, in recent years, has been developing more and more in various directions. Despite it being a problem for the protection of public health and a socio-economic scourge, it is also an issue of forensic nature. The expansion of this phenomenon is due to the constant production of new substances, chemically similar to known ones or completely new, designed to bypass the rules about psychoactive drugs which are in force in different countries. In this way, NPSs keep appearing on the market in a totally legal way. This brings the Police and other scientific bodies to have to constantly keep up to date on new drugs on the market, to know them from a chemical and behavioral point of view, to identify them and to develop analytical techniques to recognize and quantify them. [1]
This work was intended to study the phenomenon of the diffusion of NPSs, in order to create a database containing the greatest possible number of NPSs currently available on the internet. Moreover, its focus was to specifically characterize, through a high resolution mass spectrometer (with Orbitrap technology), different NPSs which were purchased online or sequestered by the Carabinieri of RIS.
Furthermore, a study was conducted on MT-45 in order to characterize this substance in the urinary matrix and to identify its metabolites, with the purpose to assess the substance intake even after a long time after the assumption. In this way, we could verify that MT-45 is not metabolized and after a certain time it is excreted without any modification in the urine. [2]
Lastly, a qualitative and quantitative screening method for MT-45 in the urinary matrix was set up.

References

[1] United Nations Office on Drugs and Crime, *World Drug Report 2015,* United Nations publication, Sales No. E.15.XI.6, (2015).
Hajime Fujimura, Kaiko Tsurumi, Masakatsu Nozaki, Mikio Hori, Eiji Imai, Analgesic activity and opiate receptor binding of 1-cyclohexyl-4-(1,2-diphenylethyl)piperazine, Journal of Pharmacology, Vol. 28, No. 3. (1978)

Identifying non-locals in the Italian Copper Age cemetery at Pantano Borghese (Rome).

Marco Ferrante [1,2,3], M. Peruzzo [1], S. Nisi [2], M.L. Di Vacri [2], F. De Angelis [3], P.R. Trincherini [2], M. Angle [4], C. Cavazzuti [5]

[1] Isotopic Ratio Institute S.r.l. info@isotopicratio.com
[2] Laboratori Nazionali del Gran Sasso- INFN - Laboratorio di Spettrometria di Massa Isotopica nisi@lngs.infn.it
[3] Università degli studi dell'Aquila francesco.deangelis@univaq.it
[4] Soprintendenza per i Beni Archeologici del Lazio micaela.angle@beniculturali.it
[5] Museo Nazionale Preistorico Etnografico "Luigi Pigorini" claudio.cavazzuti@gmail.com

The isotopic ratio of strontium $^{87}Sr/^{86}Sr$, measured by Thermal Ionization Mass Spectrometry (TIMS), was used to highlights the mobility patterns of an Italian community of the 3rd millennium BC. In nature strontium has four stable isotopes: ^{88}Sr, ^{87}Sr, ^{86}Sr and ^{84}Sr. Three of them (^{88}Sr, ^{86}Sr and ^{84}Sr) are non-radiogenic, while ^{87}Sr is the product of the spontaneous β-decay of ^{87}Rb, which turns into ^{87}Sr with a half-life T 1/2 of ~ $4.7x10^{10}$ y. The strontium isotopic composition in soils depends therefore on the age and on the composition (Sr/Rb ratio) of the soil. Variations in the Sr/Rb system can generate differences in the $^{87}Sr/^{86}Sr$ ratio both on a global and local scale. These small differences can be used to determine the geographical origin of individuals, given that biological processes, such as metabolism, do not significantly fractionate strontium isotopes[1] and that the $^{87}Sr/^{86}Sr$ ratios on samples reflect the sources of strontium available during their formation[2]. In this study 13 human teeth and 4 samples of local markers (fauna and geology) from Pantano Borghese have been analyzed. The Copper Age cemetery at Pantano Borghese (Rome) has been chosen for its significant quantity of archeological and anthropological data, as well as for the good preservation of the human and faunal odontoskeletal remains.

References

[1] G. Fortunato, K. Mumic, S. Wunderli, L. Pillonel, J. O. Bosset and G. Gremaud, Application of strontium isotope abundance ratios measured by MC-ICP-MS for food authentication, *J. Anal. At. Spectrom.*, **19**, 227-234 (2004)

[2] E. J. Dasch, Strontium isotopes in weathering profiles, deep-sea sediments, and sedimentary rocks, *Geochim. Cosmochim. Acta,* **33**, 1521-1552. (1969)

Molecular Markers in Biological Fluids by MRM Mass Spectrometry.

Anna Illiano, Francesca Pane, Valentina Arpino, Piero Pucci and Angela Amoresano

Dept. of Chemical Sciences, University of Naples Federico II, Naples, Italy 081674050

Forensic Science is based on the application of scientific techniques and methodologies to the traditional judicial investigations, in connection with the investigation of a crime or social behaviour. In forensics investigations, DNA testing is of fundamental importance to recognise the individuals involved in a crime but it is not specific to distinguish different body fluids. On the other hand, the presence of certain body fluids can be used as excellent indicator of the sequence of events which occurred. The most common body fluids found at crime scenes are blood, semen, saliva, urine, and vaginal fluid. For more than a century, numerous types of body fluid identification methods have been developed, such as chemical tests, immunological tests, protein catalytic activity tests, spectroscopic methods and microscopy. However, these conventional body fluid identification methods are not highly specific, and they need larger amounts of sample to conduct a single analysis. There is a need for a universal confirmatory test that can be applied to an unknown stain which will be able to identify any of the body fluids that might be present.[1] Since body fluids have evolved to perform different functions, they contain within them different proteins, or different levels and combinations of proteins that give each body fluid a unique protein signature that can be used to distinguish one body fluid from another. For this reason, an alternative to "traditional" test could be a proteomic approach based on the use of tandem mass spectrometry with faster, simpler analysis. The aim of this project concerns the development and optimization of an universal method based on Mass Spectrometry Multiple Reaction Monitoring (MRM) for the identification of protein biomarker that are highly specific for four different body fluid: blood, saliva, semen and urine. The protein fraction has been extracted from each body fluids and it has been identified by mass spectrometry methodologies: nano-LC/MS-MS. The results obtained have been compared with literature data and unique protein (biomarkers) have been selected. Then, for each protein biomarker, MRM has provided for the selection of the specific tryptic peptides, said proteotypics, as representatives stechiometric of proteins to be analyzed by the use of bioinformatics software (eg. Skyline). Methods' optimization has been carried out using a triple quadrupole instruments (XEVO-TQS, Waters) in MRM ion mode. The first analyzer only selects the values of m/z relative to the precursor ions, the second acts as a collision cell (CID) fragmenting the precursor and the third identifies the specific fragment. Finally, once optimized this method was applied to a real samples on various supports such as cloth, wood, plastic, plaster and paper. Thus leading to the unique identification of each body fluid.

References

[1] Analysis of body fluids for forensic purposes: From laboratory testing to non-destructive rapid confirmatory identification at a crime scene. Virkler K., Lednev I.K.; *Forensic Science Int.* **188**, 1–17(2009).

Evaluation of pharmacokinetic profile of the "new psychoactive substance" AH-7921 and analysis of its *in vivo* metabolites, by high-resolution mass spectrometry.

Jacopo Lucchetti [1], Alice Passoni [2], Claudio M. Marzo [3], Luigi Cervo [3], Renzo Bagnati [2] and Marco Gobbi [1]

[1]Department of Biochemistry and MolecularPharmacology, IRCCS Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy.
jacopo.lucchetti@marionegri.it
[2] Department of EnvironmentalHealthSciences, IRCCS Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy.
[3] Department of Neuroscience, IRCCS Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy.

AH-7921 is a synthetic opioid added to schedule I of the Single Convention on Narcotic Drugs in 2015. Several metabolites were found in blood and urine of AH-7921 consumers and in *in vitro* studies. Brain and plasma samples of rats treated intraperitoneally were analyzed by high performance liquid chromatography coupled with high-resolution mass spectrometry (HPLC-HRMS), operating in Full MS and MS/MS scan. Determination of drug concentrations and semi-quantitative analysis of the main metabolites were obtained by an HPLC-SRM validated method.
Different metabolites (N-demethylated, N-didemethylated, hydroxylated, N-demethylated hydroxylated and N,N-didemethylated hydroxylated) were identified in plasma and brain samples.
The exact mass of the fragments, combined with the chromatographic separation, allowed to detect different isomers of the hydroxylated metabolites. AH-7921 C_{max} was reached 30 min after treatment, both in plasma and brain (with a brain-to-plasma ratio of 16.6).These levels declined with a similar $t_{1/2}$ of about 3 hours. Results from semi-quantitative analysis showed that the N,N-didemethylated metabolite was slowly eliminated from brain tissue compared to the parent compound.
In conclusion, the HPLC-HRMS analysis allowed the identification of several metabolites in plasma and brain deriving from demethylation, hydroxylation and combinations of these reactions. Moreover, quantitative analysis showed that AH-7921 and its main metabolites, readily reached the brain with brain-to-plasma ratios of ~15-20.

Non-targeted LC-MS based metabolomics analysis of the urinary steroidal profile.

Amelia Palermo [1,2,3], Francesco Botrè [3,4], Xavier de la Torre [3], Nicola Zamboni [2]

[1] Department of Chemistry and Pharmaceutical Technologies, Sapienza University of Rome, Italy
[2] Institute of Molecular Systems Biology, ETH Zurich, Switzerland
[3] Laboratorio Antidoping, Federazione Medico Sportiva Italiana
[4] Department of Experimental Medicine, Sapienza University of Rome, Italy

The urinary steroidal fraction has been extensively explored as non-invasive alternative to monitor pathological conditions as well as to unveil the illicit intake of pseudo-endogenous anabolic steroids in sport. However, the majority of previous approaches involved the *a priori* selection of potentially relevant target analytes. Here we describe the non-targeted analysis of the urinary steroidal profiles. The workflow includes minimal sample pretreatment and normalization according to the specific gravity of urine, a 20 min reverse phase ultra-performance liquid chromatographic separation hyphenated to electrospray time-of-flight mass spectrometry. As initial validation, we analyzed a set of quality control urines spiked with glucurono- and sulfo- conjugated steroids at physiological ranges. We then applied the method for the analysis of samples collected after single transdermal administration of testosterone in hypogonadal men. The method allowed profiling of approximately three thousand metabolic features, including steroids of clinical and forensic relevance. It successfully identified metabolic pathways mostly responsible for groups clustering even in the context of high inter-individual variability. It also allowed discovering novel metabolic features correlating with testosterone administration. These outcomes set the stage for future studies aimed at implementing currently monitored urinary steroidal markers both in clinical and forensic analysis.

In silico prediction of 4,4'-DMAR metabolism and *in vivo* confirmation in rats by high resolution mass spectrometry.

Alice Passoni [1], Jacopo Lucchetti [2], Claudio M. Marzo [3], Luigi Cervo [3], Marco Gobbi [2] and Renzo Bagnati [1]

[1]Department of EnvironmentalHealthSciences, IRCCS Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy.
alice.passoni@marionegri.it
[2] Department of Biochemistry and MolecularPharmacology, IRCCS Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy.
[3] Department of Neuroscience, IRCCS Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy.

4-methyl-5-(4-methylphenyl)-4,5-dihydrooxazol-2-amine (4,4'-DMAR) is an analog of the psychostimulants 4-methylaminorex and aminorex, recently added to the schedule II of 1971 Convention on Psychotropic Substances. No information is available about the drug's pharmacokinetic profile and its metabolites.
In the present study, metabolism profilers available within OECD QSAR ToolBox were used to identify its potential metabolites. The predicted ones were searched in plasma and brain of rats treated intraperitoneally. Analysis were performed by high performance liquid chromatography - high resolution mass spectrometry (HPLC-HRMS), operating in MS/MS mode.
The predicted hydroxylated, oxidated, hydrolysed and deaminated metabolites were found in plasma and brain samples. MS/MS spectra and chromatographic separation confirmed the structure of metabolites and identified different isomeric forms of the hydroxylated ones.
Pharmacokinetic profile of 4,4'-DMAR obtained by a validated HPLC-SRM method showed a rapid absorption (15 min) and a rapid clearance in plasma. Analysis of brain tissues showed that drug levels reached C_{max} at 30 min after treatment and then declined as plasma levels.
In parallel, semi-quantitative analysis were performed for the hydroxylated and oxidated metabolites and results suggested a low brain uptake of these main metabolites.
In conclusion, the HRMS analysis, associated with the *in silico* prediction, allowed the identification for the first time of the *in vivo* metabolites of 4,4'-DMAR. Moreover, investigation of their pharmacokinetic profiles in plasma and brain showed that 4,4'-DMAR (but not its main metabolites) readily reached the brain with a high brain-to-plasma ratio.

Entomotoxicology: development and validation of GC-MS and LC/MS-MS methods for nicotine, metanfetamine, endosulfan and coumatetraryl detection in blowflies.

Marco Pazzi [1],Paola A. Magni [2], Ian R. Dadour [3], Marco Vincenti [1]

[1] *Dipartimento di Chimica, Università di Torino, Via P. Giuria n.7 Torino, Italy*

[2] *Department of Molecular and Biomedical Sciences, School of Veterinary and Life Sciences, Murdoch University, Western Australia*

[3] *Program in Forensic Anthropology, Department of Anatomy & Neurobiology, Boston University School of Medicine Boston, MA-USA*

In addition to their use in the estimation of the minimum post-mortem interval (minPMI) necrophagous blowflies (Diptera: Calliphoridae) may also represent a reliable specimen for toxicological analyses (Entomotoxicology) especially in the absence of tissues and fluids normally taken for such purpose. Numerous researchers have extracted several toxicological substances and measured their effects in altering the morphology, the rate of development and the survival on blowfly immatures associated with remains. The accumulation of such substances in blowflies may compromise their use for the estimation of the minPMI.
At present a modest number of substances and insect species/instars have been studied. Furthermore, many early studies utilized analytical procedures which are now obsolete with little/no validation.
This research presents the method validation and the effects on blowflies for:
Nicotine in Calliphora vomitoria (L.) by GC-MS. Nicotine is an alkaloid present in the tobacco plant. Nicotine is one of the most deadly poisons known to man and it's extremely easy to purchase.
Metanphetamine in C. vomitoria by GC-MS. Metanphetamine is a psychostimulant synthetic drug originally used as a medical treatment but now a common recreational drug (meth, crystal, and ice). High doeses can cause death.
Endosulfan in C. vomitoria by GC-MS. Endosulfan is a toxic pesticide, responsible for many fatal poisoning incidents around the world. Endosulfan is acutely neurotoxic to both insects and humans.
Coumatetralyl in Lucilia sericata (Meigen) by LC/MS-MS. Cumarine compounds are used in anti-coagulant therapies. Coumatetralyl is a product largely used in poisoned baits.