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LE INDAGINI FORENSI ED IL CONTRIBUTO DELLA SPETTROMETRIA DI MASSA

FORENSIC INVESTIGATIONS AND THE CONTRIBUTION OF MASS SPECTROMETRY

Roma, 1 marzo 2019
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Scientific Program

8.30 - Registration of participants

Introduction, chairs: D.T.S. Serena DETTI, Manuel SERGI

Greetings from Authorities

9.20 – 9.30	**Vittorio RIZZI** – *The Head of Central Anticrime Directorate*
9.30 – 9.40	**Fausto LAMPARELLI** - *The Head of Forensic Science Police Service*
9.40 – 9.45	**Donatella CARUSO** – *President of Mass Spectrometry Division of SCI*
9.45 – 9.50	**Claudio MINERO** – *President of Analytical Chemistry Division of SCI*
9.50 – 10.20	**The role of the forensic analytical chemist from an academic perspective: the case of molecular fingerprinting** Simona FRANCESE – *Biomolecular Science Research Centre, Sheffield, UK*
10.20 – 10.40	**From the collection of evidences on the crime scene to the analysis: identification of benzodiazepines in drug-facilitated crimes** D.T.S. Anna Maria CAPUTO *Central Anticrime Directorate of Italian National Police, Forensic Science Police Service (DAC-SPS), Rome*
10.40 – 11.00	**Detection of fentanyl analogues and synthetic opioids in real hair samples. A picture of the U.S. emergency** Marco VINCENTI - *University of Torino*
11.00 – 11.05	**Illicit administration of estradiol in cattle: case report** Marilena GILI – *IZS of Piemonte, Torino*
11.05 – 11.10	**Use Of SPME coupled with Mass Spectrometry to assess the use of self defence pepper spray** D.T.S. Morela STRANO *Int. Forensic Science Police Office of Torino*
11.10 – 11.30	*Coffee Break*

Session 1, chairs: C.C.T. Sabino NAPOLETANO, Donatella CARUSO

11.30 – 11.50	**Forensic analytical chemistry: the activities of police forces** Egidio LUMACA, *Expertise in Forensic Science Police Service, Rome*
11.50 – 12.05	**Solving "Cold Cases" by HRMS Toxicology Screening** Vincent DI FAZIO - *National Institute of Criminalistics and Criminology, Bruxelles*
12.05 – 12.20	**Untargeted investigation of NPS: UHPLC-HRMS analysis and Cloud-MS-database comparison** Ten. Col. Adolfo GREGORI - *Forensic science laboratories of Carabinieri Force (RaCIS), Rome*
12.20 – 12.35	**Near-Infrared hyperspectral imaging (NIR-HSI): a powerful tool in forensic sciences** Paolo OLIVERI – *University of Genova*
12.35 – 12.50	**Application of LC-ESI-MS/MS to the determination of illicit drugs and medicines in blood samples in the context of the Italian National Street Code Art. 187.** Marco AGOSTINI – *Toxicology laboratory – A.S.U.R. AV1 –Pesaro*
12.50 – 13.05	**Uncovering the past: the power of GC-TOF and GCxGC TOF** Marcello MANFREDI – *University of Piemonte Orientale, Alessandria*

13.05 – 13.10 **Investigations and analysis of cocaine from clandestine "laboratory"**
D.T.S. Guido PERSICO – *Interregional Forensic Science Police Office of Naples*

13.10 – 13.15 **White Spirit, Black Widow (in the land of Red Chicory)**
D.T.S. Gabriele GRIGOLI – *Interregional Forensic Science Police Office of Padova*

13.15 – 14.45 **Business Lunch – Poster Session**

Session 3, chairs: D.T.S. Damiano RICCI, Emanuela GREGORI

14.45 – 15.00 **Home-made explosives: analytical approaches and case reports**
D.T.S. Paolo ZACCHEI - *Central Anticrime Directorate of Italian National Police, Forensic Science Police Service (DAC-SPS), Rome*

15.00 – 15.15 **An ocfentanil-related death case: UHPLC-MS analysis of the drug and its metabolites**
Marica ORIOLI - *Laboratory of Forensic Toxicological, University of Milan*

15.15 – 15.30 **Untargeted approach and retrospective analysis: new opportunities for forensic investigations**
Rossana SCARPONE - *IZS dell'Abruzzo e del Molise, Teramo*

15.30 – 15.45 **MS Investigation on Designer Drugs by Retrosynthetic Analysis**
Cap. Giuliano IACOBELLIS - *Forensic science laboratories of Carabinieri Force (RaCIS), Rome*

15.45 – 16.00 **Desorption electrospray ionization-high resolution mass spectrometry for screening the presence of new psychoactive substances in oral fluid**
Federica BIANCHI - *University of Parma*

16.00 - 16.05 **Indoor illegal grow marijuana site, with low THC levels**
D.T.S. Morela STRANO - *Interregional Forensic Science Police Office of Torino*

16.05 – 16.10 **Trace Evidence Analysis: Forensic examination of fibres**
C.C.T. Elena LUCATELLI - *Forensic Science Police Service (DAC-SPS), Rome*

16.10 – 16.30 **Tea Break**

Session 4, chairs: C.C.T. Elena LUCATELLI – Alessandro GIUFFRIDA

16.30 – 16.45 **The future perspectives of mass spectrometry: an ever-wider horizon with use in clinical emergencies of toxicological interest**
Elia DEL BORRELLO - *Expertise in Forensic Toxicological Lab, Legal Medicine Office, Bologna*

16.45 – 17.00 **Synthetic isoflavones and doping: influence on the urinary steroid profile studied by gas chromatography – mass spectrometry**
Michele IANNONE - *FMSI Antidoping Laboratory, Rome*

17.00 – 17.15 **A novel approach for the determination of OGSR by means UHPLC-HESI-HRMS**
Flaminia VINCENTI - *Sapienza – University of Rome*

17.15 – 17.30 **New Psychoactive Substances: metabolite identification by means complementary approach**
Federico FANTI - *University of Teramo*

17.30 – 17.40 **Conclusions**

Oral Presentation

The role of the forensic analytical chemist from an academic perspective: the case of molecular fingerprinting

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As criminals become more forensically aware of their traceability, forensic scientists need to up their game with advancing implementable technology. Technology improvements should aim to enhance recoverability of the evidence and expand, strengthen and communicate retrievable intelligence.

Pioneering work at Sheffield Hallam University (UK) has demonstrated the capabilities of Matrix Assisted Laser Desorption Ionisation Mass Spectrometry Imaging (MALDI MSI) to yield both physical and chemical information by providing multiple images of the same fingermark, simultaneous with additional intelligence[1]. Physical information could complement the ridge pattern retrieved by CSI or even provide the only ridge pattern image for database comparison[1], as well as enabling separation of overlapping impressions[2]. The opportunity to detect chemical information (aminoacids, fatty acids, peptides, proteins, drugs, toiletry products, condom lubricants and blood as a few examples) could provide investigative leads on the suspect's lifestyle and activity prior to leaving the mark as well as being useful to prove/disproof the suspect's statements[3]. Consumption of drugs and alcohol can also be proved and this is useful to inform on the suspect's state of mind while committing the crime[4].
Using molecular fingerprinting pioneered at Sheffield Hallam University, this presentation aims to illustrate the role and the challenges of an analytical chemist within their efforts in the delivery of implementable forensic science.
This presentation will demonstrate the power of breaking down barriers (geographical, political or of other nature) to enable collaborative and cross-disciplinary work leading to science with an impact in the real world of forensic investigations.

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From the collection of evidences on the crime scene to the analysis: identification of benzodiazepines in drug-facilitated crimes

Anna Maria Caputo[1], Serena Detti[1], Sabino Napoletano[1], Simona Carboni[1] Flaminia Vincenti[2], Manuel Sergi[3], Roberta Curini[2]

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Keywords: *benzodiazepine – drug-facilitated crimes*

Scientific research is a keystone for forensic investigations, to improve performance and to offer new and updated tools to the security services. In this work two analytical methods have been developed in GC-MS and LC-MS/MS, both based on the same sampling procedure. These methods have been designed to be applied to operational scenarios in which the determination of psychotropic and/or narcotic substances in various substrates is crucial for investigations. The so-called drugs-facilitated crimes range from robberies (often against older people) to sexual assaults and the use of benzodiazepines to alter or cancel the victim's will is frequent, so analytical target was fixed to a panel of 13 benzodiazepine, among the most common and widespread.

The proposed sampling procedure involves the use of single-use disposable devices (Alco Prep®). Sampling was performed by swabbing surface of different items such as cups, glass or plastic cups, teaspoons that could be potentially found on the crime scene. Pre-treatment optimization was carried out by testing various solvents and swabbing procedures to obtain quantitative recoveries of the analytes. A mixture of methanol/2-propanol mixture 90: 10 (v:v) turned out to be a good compromise that allowed to have recoveries close to 100% for most substances avoiding negative consequences in the chromatographic efficiency: so the extract is filtered and directly injected into chromatographic systems.

For the LC-MS/MS analysis different columns, solvents and gradients were tested to obtain best separation of analytes, achieved with the use of methanol/acetonitrile 50/50 (v/v) 0.05% HCOOH and water and a core shell chromatographic column XB-C18 (2.1 x 100 mm, 2.6 μm). Two MRM transitions for each analyte were selected to ensure a satisfactory number of identification points and the forensic identification of analyte.

GC-MS was performed with the use of MS database for the identification of benzodiazepine and also using Agilent Qualitative Navigator e UnKnowns Analysis software. Chromatographic conditions were optimized modulating from literature (1).

The proposed methods were validated and compared in terms of analytical performances. GC-MS method shown to be suitable for the analysis of different benzodiazepine even outside the target analytes, thanks to the specific databases held by Forensic Science Police Service; however, limits of detection are of the order of μg/mL without derivatization and pre-concentration steps. LC-MS/MS method shown best sensitivity: limits of detection for the analytes were below ng/mL; in this case, validation was performed according to SWGTOX guidelines for further application of method to biological samples.

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Detection of fentanyl analogues and synthetic opioids in real hair samples. A picture of the U.S. emergency

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Keywords: *Fentanyl, Synthetic Opioids, Overdose*

Novel synthetic opioids include various analogues of fentanyl and emerging non-fentanyl compounds with different chemical structures, such as AH-7921, MT-45, and U-47700. In recent years, these drugs have rapidly emerged on the drug market, and their abuse has been increasing worldwide. The motivations for use of these new compounds include their legal status, ready availability, low cost, users' curiosity or preference for their particular pharmacological properties, and the intention to avoid detection. Furthermore, more common drugs like heroin are now increasingly being replaced or cut with fentanyl or new designer opioids; thus, many drug users are unintentionally or unknowingly using synthetic fentanyl analogs. In this scenario, the detection of new psychoactive substances in hair can provide insight into their current diffusion among the population and social characteristics of these synthetic drug users.

In this presentation, we describe a simple, fast, specific and sensitive UHPLC-MS/MS method able to detect 13 synthetic opioids (including fentanyl analogues) and metabolites in hair samples. Furthermore, the method includes the detection of 4-anilino-N-phenethyl-piperidine (4-ANPP), which is considered both a precursor and a metabolite of several fentanyl analogues. The method was applied to 293 real hair samples collected in New York City (34 samples), other location throughout U.S. and Canada (159) and recovery centers (100 samples) from subjects who had reported past-year nonmedical opioid and/or heroin use. Several samples were donated from attendees of music festivals and other electronic music events. The different geographic origin of the samples allowed us to draw important epidemiologic deductions, in terms of diffusion and personal habits.

Among the 293 samples, 55% turned out positive to oxycodone, 72% to hydrocodone, 67% to tramadol, 68% to fentanyl, 53% to norfentanyl, 50% to 4-ANPP, 37% to acetyl fentanyl, 30% to furanyl fentanyl, 8% to U-47700. Three samples was positive to carfentanyl, i.e. the most potent among synthetic opioids. Quite clearly, multiple positive results were observed for most samples. Notably, 4-ANPP is a metabolite common to most fentanyl analogues, showing for the first time that this compound can be selected as a marker for several target analytes, upon hair testing. In conclusion, this study confirmed the increasing diffusion of new synthetic opioids and "fentalogs" with high potency among nonmedical opioid users, and justify the emergency situation in U.S. with 20,000 overdose cases in 2016.

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The forensic chemistry in the public administration. The activity of police forces.

***Egidio Lumaca*[1]**

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Police officers with a background in chemistry are civil servants in the public administration and judiciary police. They participate in the forensic governance of forensic science institutes, or other public laboratories, and act in agreement with prosecutors or judges. These scientists perform their activity according to the rules of the public administration and obey the rules of the criminal law.

A project concerning profiling of the main drugs is illustrated where you need a perfect equilibrium between the judiciary authority/police and scientific interests. The aim of the project is to establish a correlation between production sites of drugs and the inorganic fingerprint got by neutron activation analysis (NAA). Laboratories of police forces performed traditional analyses by GC-FID and GC-MS to find further correlation and evaluate the consistency of organic and inorganic fingerprints.

This one is a typical case where you need an effort to appease judiciary interests and police and scientific ones.

The way to solve the dualism between judiciary and administrative duties of the forensic chemist is a correct forensic governance approach. Different solution can be proposed; the road map that Italian Polizia Scientifica (Forensic Science Police Service) is pursuing the quality assurance approach of ISO 17025. This one can be integrated with the new standard ISO 9001:2015 and the risk-based thinking, consistent with the approach of ISO 17025.

Solving "Cold Cases" by HRMS Toxicology Screening

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Keywords: *Q-TOF, Toxicology, Screening*

Objectives: The daily work of a forensic toxicology laboratory is often a mix of different types of judicial cases: ante-mortem cases such as Driving Under the Influence of Drugs cases (DUID) or Drug Facilitated Sexual Assault cases (DFSA), and post-mortem cases. Regarding the type of the case, different analytical strategies using comprehensive drug screening methods, but also target methods for confirmation and quantification are applied. New Psychoactive Substances (NPS) and their rapid turn-over pose a huge challenge for forensic toxicological laboratories as the comprehensive drug screening now has to be associated with high sensitivity, but also specificity. Moreover, frequent unavailability of reference standards makes method development even more complicated. The aim of this presentation is to demonstrate the benefit of HRMS in our work of forensic toxicologists with some real cases.
Methods: The comprehensive drug screening uses a UPLC-Xevo-G2-XS-QTOF (Waters, Manchester, UK) with UNIFI software. The pre-analytical step is a protein precipitation using 100 µL of sample and 400 µL of ice-cold acetonitrile. The extracted sample is injected in negative and positive electrospray ionisation.
Results: Different cases demonstrate that this technology is of major interest in cases of low blood sample volumes and also demonstrate a very high sensitivity in comparison with traditional screening methods such as UPLC/DAD or GC-MS. The sensitivity is crucial for DFSA cases. In post-mortem cases, the most important matrix is blood but unfortunately, not always available. In these cases, the use of HRMS screening in vitreous humor - often available in very low volume - can be an interesting alternative way. Finally, the last cases reveal the detection of NPS (Naphyrone, 25B-NBOME or 4-MEC).
Conclusion: We are convinced that this technology is essential in a toxicological laboratory. While the system can both screen and quantify, our strategy is to screen with the HRMS system and to confirm and quantify the Q-TOF identified compounds, depending on the case context, by a MS/MS technique. The UNIFY software is powerful and allows us to realize a high-throughput, specific and sensitive toxicology screening with a fast interpretation of the data.

Untargeted investigation of NPS: UHPLC-HRMS analysis and Cloud-MS-database comparison

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Keywords: *NPS, UHPLC-HRMS, Database*

In recent years there has been a huge proliferation of the New Psychoactive Substances (NPS). Often the use of these substances is not recognized because the common tests do not include screening for this class of psychotropics [1]. For this reason, it has become fundamental to have the suitable tools for their identification and characterization as well as for the quantitative determination in biological matrices. The difficulty of NPS identification is the bottleneck for both the scientific community and the police forces. Despite the development of new technology and the introduction in the laboratories of the high-resolution mass spectrometry (HRMS), which provides exact mass measurements, it is still difficult to identify an unknown substance. For this reason, a significant contribution to both research and justice fields is given by the possibility of comparing mass spectra obtained in high resolution with the most common and widespread libraries of online spectra [2]. Thanks to the analyses conducted in the Full-dds acquisition mode and to the use of these software, it is possible to calculate the most probable molecular formulas and, by applying the basic fragmentation rules, it is possible to propose chemical structures for the substances found [3]. This allows the discovery of analogues of NPS not yet identified in the market and, in the case of biological samples from abusers, also of unknown metabolites [4]. This approach allows to carry out a single injection of the sample in the UHPLC-HRMS system and to obtain a complex and complete datum which can also be used for post-examination.

To date, this type of analytical approach has been used by the Arma dei Carabinieri both for the identification of NPS present in seized samples, and for the determination of drugs in biological matrices, where the amount of sample was very limited or polluted or coming from the waste of previous assessments. Several cases will be presented, including the identification of two synthetic substances such as DOC and DMA in a fragment of a stamp, identification and reporting to the National Early Warning System of a new fentanyl-derivative, the 4-fluoro-Fu-F. Finally, it will be presented how this approach has been fundamental for the determination of several drugs present simultaneously in an unrepeatable sample obtained by treating and analysing the laboratory waste obtained from the DNA determination and the residue of a PSA-test.

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NEAR-INFRARED HYPERSPECTRAL IMAGING (NIR-HSI): A POWERFUL TOOL IN FORENSIC SCIENCES

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Keywords: *near-infrared spectroscopy; hyperspectral imaging; non-destructive analysis*

During the last decade, there has been an increasing interest in the development of customised analytical methods based on non-destructive chemical imaging in several fields of application. This is of particular interest when it is important to spatially visualise the results as an unequivocal proof of a crime and without damaging the evidences, because further investigations could be required during legal inquiries. For these reasons, the present work aims at developing a reliable method for the application of hyperspectral imaging in the near infrared region (HSI-NIR) to detect biological traces on the crime scene; in this way, a screening procedure is proposed to detect areas of interest in which collecting micro-samples for applying the conventional more targeted DNA analyses. In more detail, this work will give a fundamental contribute to forensic scientists in identifying biological traces directly on specimens, recognising the type of trace (e.g. blood, urine, semen and their mixtures as well as food traces) regardless of the material constituting the collected evidences (e.g. paper, glass and cloth).

HSI-NIR is one of the newest technologies developed in the field of NIR spectroscopy; it is based on the interaction between radiation – in the region of the electromagnetic spectrum between 1000 and 2500 nm – and the sample, to obtain chemical information about its composition, thanks to the spectroscopic absorption of characterising compounds. The advantages of this approach are exploited when samples to be evaluated are characterised by a non-uniform distribution of chemicals inside the whole matrix. In this situation, the validity of traditional chemical analyses is strictly dependent upon the design of a correct sampling plan; the extent of sampling required to account for such a variability of distribution is necessarily quite large and the method of collecting samples is also critical. All these limits become serious when data collected have to be used for drawing conclusions at the service of law. In fact, sampling limitations leave an analytical crack in which crime may act.

Often, HSI-NIR equipment setup is not directly applicable, since optimisation steps are required, in terms of both instrumental parameters and image acquisition; furthermore, the acquisition of images is not sufficient for extracting reliable information from samples in a robust way. For these reasons, in this work, HSI-NIR is supported by a proper chemometric strategy particularly suited to account for the spatial information, allowing to define a customised method for the specific application.

Application of liquid chromatography with electrospray tandem mass spectrometry to the determination of illicit drugs and medicines in blood samples in the context of the Italian National Street Code Art. 187.

Marco Agostini[1], Caterina Renzoni[1] and Lucia Alfieri[1]

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Keywords: *UHPLC-MS/MS, New psychoactive substances (NPSs), DRUID*

Driving under the influence of illicit drugs (DRUID) in Italy is regulated by the article 187 of the National Street Code (NSC), this regulation forbids to drive with physical and mental impairment due to drugs intake.
However, the verification of infringement of Art. 187 of the NSC is a very complex matter and historically it is a challenge for Analysts and Toxicologists due to a number of reasons, including the wide variety of substances to research (NPSs, prescription drugs), the lack of threshold values to be applied and for the presence of many substances for which the relationship (blood concentration vs impairment degree) has not been demonstrated yet. Therefore, any concentration of psychoactive compounds or their active metabolites in blood (or oral fluid) may lead to a legal prosecution. [1,2]
The recent introduction of the "Road Homicide" within the Penal Code (Law n.41 March 2016) which leads to increase the severity of penalties has made scenario much more delicate.
In this context, forensic laboratory plays a key role. Toxicological tests on biological matrix shall be based on two different analytical steps, the preliminary test or screening test and the confirmatory test. [3]
A common laboratory approach in routine analysis is to perform a fully-automated, preliminary assay, such as an immunoassay, followed by confirmatory mass spectrometry method.
The immunoassay is a cost-effective tool that plays an useful role in screening the presence of certain drug classes, for instance opiates or benzodiazepines, but not the identification of specific compounds (Morphine, Alprazolam). It is well known that the use of immunoassay tests to detect the presence of classic illicit substances often yields false negative or false positive results, furthermore immunoassay cannot detect other psychoactive compounds, such as NPSs. [4]
Mass spectrometry coupled with chromatography (GC-MS, LC-MS, LC-MS/MS, LC-HRMS), is the most frequently used technique to confirmatory quantification test, because it provides both sensitivity and specificity.
Currently, a growing number of applications using tandem mass spectrometry as preliminary screening test have been reported. [5,6]
Here we present a rapid targeted method for qualitative screening of 75 DRUID significant compounds in whole blood by UHPLC-MS/MS that was developed and validated by the Toxicological Laboratory of Pesaro. Samples were processed applying a protein precipitation approach using cold acetonitrile, after centrifugation, aliquot of supernatant was evaporated under a stream of nitrogen and reconstituted with 9:1water/methanol. Chromatographic separation was achieved in 8 min using Hypersil GOLD PFP LC Column (50 x 2.1 mm, 1.9 μm).
The MS/MS mass spectrometer operated using positive ionization using multiple reaction monitoring (MRM) data dependent acquisition. Twenty different deuterium-labelled internal standards were included to ensure the reliability for the analysis of different classes of drugs.
The method validation protocol includes selectivity, carryover, recovery, matrix effect and limit of detection. The total workflow time from sampling to data acquisition was less than 45

minutes. Method validation demonstrates that this protocol works reliably and is able to provide accurate results.

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Uncovering the past: the power of GC-TOF and GCxGC-TOF

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Keywords: *Gas chromatography-mass spectrometry; Paleo-metabolomics; Archaeometry*

Gas chromatography-mass spectrometry (GC-MS) is particularly useful for the analysis of both low-polarity and high-polarity molecules. Limitations in resolving power of GC-MS have been improved by the development of two-dimensional workflows combined with mass spectrometer. In this research, a GC-TOFMS (Pegasus BT, Leco) and a GCxGC-TOFMS (Pegasus BT 4D, Leco) were used for the untargeted analysis of non-invasive samples obtained from the ancient Egyptian mummy of Nebiri and from a painting from Leonardo da Vinci. Nebiri was an ancient Egyptian dignitary who lived 3500 years ago under the reign of Thutmoses III (1479–1424 BC; 18th Dynasty). His tomb was discovered by E. Schiaparelli in 1904 in Luxor and only the head and the canopic jars containing the internal organs (lung, stomach, liver, and intestines) were preserved. Naturally preserved and embalmed bodies from archaeological contexts represent a powerful source of information. The metabolomics analysis revealed the embalming recipe but also the chemical composition of the lung.

The same metabolomics approach was used for the analysis of the painting "Donna Nuda" by Leonardo da Vinci, which is conserved at the Hermitage museum in St. Petersburg. The functionalized film technology coupled to gas chromatography analysis was used to explore the surface of the painting in order to ascertain the techniques used in its drawing. The original artistic technique of the old master was then deciphered for the first time.

The high sensitivity of GC-TOFMS and the high resolving power of GCxGC-TOFMS were fundamental for the identification of the embalming recipe and the uncovering of Leonardo artistic technique.

HOME-MADE EXPLOSIVES: ANALYTICAL APPROACHES AND CASE REPORTS

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Keywords: *precursors, improvised explosives devices, homemade explosives*

The use of explosives precursors to make home-made explosives is a widespread practice in Italy and Europe. The Regulation of the European Parliament[1] on explosives precursors regulates marketing of some substances widespread in EU.
Explosives precursors and Home-Made Explosives (HME) analyses are made with several instrumental techniques in order to characterize organic and inorganic components[2].
In two cases, which have strongly affected public opinion, have been used Improvised Explosives Devices (IED): the HME were made with explosives precursors easily available on the market. The first terrorist attack took place in Milan in 2009, at the entrance of the military barrack Perrucchetti, the second one in Brindisi in 2012, in front of the secondary school Morvillo Falcone.

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An ocfentanil-related death case: UHPLC-MS analysis of the drug and its metabolites

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OcF is a potent synthetic opioid, structurally analogue of fentanyl, abused as a new psychoactive substance in the recreational drug scenario. This work deals with a drug-related fatality involving OcF. In order to obtain a diagnosis of death, a forensic-based method involving anamnestic, clinical and circumstantial, anatomo-pathological and toxicological criteria was used. It was known that the deceased person had a substantial anamnestic history of drug addiction when in life, which may direct the diagnosis towards a death correlate with a chemical cause. As far as the anatomo-pathological findings are concerned, limbs lesions were found to be significant and consisted with a narcotic substance injection. Indeed, the toxicological investigations performed on the brown powder and subsequently on the biological specimens, taken from the body of the deceased person, has shown the presence of OcF in the femoral and cardiac blood, in urine, bile, brain, liver, lung and kidney, together with paracetamol and caffeine. To do this, an ultra-performance liquid chromatography tandem mass spectrometry (UHPLC–MS/MS) based method was developed and validated for OcF quantitation and the simultaneous main metabolites identification. This represents the first reported case in Italy of a fatal outcome related to OcF and the first time the drug is quantified in tissues. In addition, the toxicological data obtained in the present study, taken together with the well-known potency and danger of the substance and with the congruence between anamnestic, circumstantial and anatomopathological data with the hypothesis of acute narcosis, allow us to support the lethal power of OcF.

Untargeted approach and retrospective analysis: new opportunities for forensic investigations

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Keywords: *Untargeted analysis, HRAMS (High Resolution Accurate Mass Spectrometry), forensic investigation*

Over the years, the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "Giuseppe Caporale" (IZSAM), as public health institute, has tackled lots of requests of identification of unknown substances in samples of various origin. These requests have not always been satisfied because the targeted methods traditionally applied in the laboratory needed in advance the determination of a list of molecules to be investigated.
These methods, also applied in the forensic field for the detection and the identification of substances, require the previous assumption of the unlawful act.
However, it is not always possible to have an investigative framework that allows the correct identification of molecules to be identified.
In the last years, the IT technological development has opened new unprecedented horizons in the detection and identification of unknown chemicals: High Resolution Accurate Mass Spectrometers combined with dedicated software allow to overcome limits related to targeted analysis in order to investigate the sample in a wider analytical range [1].
Our analytical method provides for a simple treatment of the sample, such as "Dilute-and-shoot" extraction followed by a non-selective chromatographic separation by using a C18 column and acquisitions in High Resolution Accurate Mass Spectrometry in ESI positive and negative modes. In particular the acquisitions in FullScan-AIF and FullScan-ddMS^2 allow not only the clear detection and identification of isobaric compounds, but also to dispose of stored data that best represent the sample fingerprint to be used in future retrospective analysis. Furthermore the differential analysis between the unknown sample and the blank sample allows to remove all the matrix molecular components and Aggregated Computational Toxicology Resource (ACToR) helps to understand and predict chemical interpretation of substances [2-3].
In order to verify that the proposed method is suitable to the scope of application, a "*Proficiency Test for unknowns in water (2018-11)"* by RIKILT has been performed successfully.

In this work a short description of the analytical method developed by our laboratory will be shown, with reference to the databases used. Finally, the following real cases of investigations will be reported:

1) *Seizure of white pills in a gym locker*: the legal authority assumed that it could be Methandienone, a synthetic hormone, traditionally used as anabolic substance. On the contrary, it was identified as Stanozolol, a drug with anabolic properties 4 to 5 times greater than Methandienone.

2) *Unknown solution with suspected occurrence of pesticides*. The sample, previously submitted by another laboratory to a target analysis for pesticide, came back negative. Instead, the results obtained by the untargeted approach identified the active substance

Glyphosate. Even if Glyphosate is an herbicide, it was not detected in the first analysis because it was not included in the target list. These different results emphasize the difference between targeted and untargeted approaches.

3) *Analytical potential of the untargeted approach applied to retrospective analysis*.
On 20th July 2017, Belgium informed the European Commission on the identification of eggs contaminated with Fipronil and a consequent community alert has been issued. Two were the possible sources of contamination: the feed and the unlawful treatment with Dega-16 against red mites. As a consequence, the Italian Ministry of Health established an ad-hoc monitoring programme to get a comprehensive view on the contamination of eggs and poultry products related to illegal uses of Fipronil **[4]**. Due to positive results, lots of companies have been seized and, over a period of 4 months, about 2000 samples have been taken by the authority and analysed for searching Fipronil and its metabolite Fipronil Sulfone. In the meantime, other suspects arose and as a consequence on 28th August 2017 also Amitraz and its metabolites have been included in the monitoring programme; on 11st September 2017 other 11 acaricides were investigated and finally, on 30th October 2017, also Fipronil Sulfide and Fipronil-desulfinyl were included in the monitoring scope. Thanks to the retrospective analysis it was possible to perform other investigations by reprocessing raw data file obtained in the first instance, so as to keep intact the scenario at the moment of the sampling without having at disposal the original sample anymore.

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MS Investigation on Designer Drugs by Retrosynthetic Analysis

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Keywords: *SPME-MS, Retrosynthesis, New Psychoactive Substances*

Retrosynthetic analysis is a strategic technique for planning the syntheses of new organic molecules with high level of complexity. This is achieved by transforming the required chemical structure (called Target Molecule, TM) into simpler precursors without assumptions regarding the required starting materials. Each precursor is examined using the same method. The goal of this method is the structural simplification by the so called “disconnection approach”. Often, a synthesis will have more than one possible synthetic route. Retrosynthesis is well suited for discovering different synthetic routes and comparing them in a logical and straightforward fashion allowing to obtain the TM. On the other hand such a robust methodology has to be considered as a powerful tool for Forensic Investigation. An example can be provided by precursor analysis in illicit drug manufacture form Clandestine Lab but also when new psychoactive substances need to be identified but no reference material or library are still available. The precursors found by trace MS analysis allow to make hypothesis on the unknown substance nature but furthermore to confirm the assumption that some other contaminants cannot be derived from synthesis but are originated, for example, by the purification step only.
In this framework, a practical application of retrosynthetic approach in forensic analysis of some designer drugs will be accounted; by means of Solid Phase Micro Extraction (SPME) methodology coupled to Gaschromatograpy/Mass Spectroscopy (GC/MS), the possibility to characterize the volatile pattern precursors in the headspace of methamphetamine crystals it will be accounted.
After the evaluation of the results, the possibility to relate a common nail solvent found during the seizure with the purified drug found in the suspect availability beyond any reasonable doubt will also be proven.

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Desorption electrospray ionization-high resolution mass spectrometry for screening the presence of new psychoactive substances in oral fluid

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Keywords: *desorption electrospray ionization; high resolution mass spectrometry; new psychoactive substances*

New psychoactive substances (NPS) are a wide group of synthetic drugs of abuse able to mimic the effects of controlled illicit drugs. Owing to their "legal status" and online availability, the use of NPS has rapidly increased over the last decade. NPS are challenging in several respects, including the analytical testing difficulties for their detection in biological samples, in clinical and forensic cases. Illicit substances are detected in body fluids like blood, and urine, but recently, oral fluid has been used as an alternative matrix because of its ease of sampling and non-invasiveness. In this context, a key issue is the development of novel screening methods able to assess the presence of the NPS at low concentration levels. Ambient ionization techniques are powerful analysis tools for the rapid screening of samples with minimal or without any sample preparation or chromatographic separation prior to analysis, thus reducing analysis time [1]. Among ambient ionization sources, desorption electrospray ionization mass spectrometry (DESI) has been widely used for forensics applications [2]. The role of new materials as sample substrates in DESI-MS analysis is of paramount importance to increase the instrumental response of the investigated analytes. In this study, a Micro Extraction by Packed Sorbent (MEPS)-DESI-HRMS method was optimized and validated for NPS (synthetic cannabinoids, synthetic cathinones, ketamine) determination.

DESI parameters affecting the ionization yield and the ion recovery were optimized in terms of: i) spray composition; ii) spray flow; iii) acquisition mode; iv) geometrical parameters. With the aim of achieving high sensitivity, different sample substrates, i.e. unfunctionalized PLA, carbon/PLA-based films and a silica-based coating were synthetized and tested. Both unfunctionalized PLA and the silica-based coating showed enhanced performances in improving the DESI-HRMS responses of the investigated compounds. A full factorial design followed by the multi-criteria method of desirability functions was applied for the optimization of the MEPS extraction in terms of both loading and eluting cycles. The method was validated obtaining detection limits in the μg/kg level, good linearity, precision and accuracy and applied for the analysis of 40 saliva samples collected during private parties from young volunteers. Finally, a confirmatory GC-MS Selected Ion Monitoring (SIM) method was developed to confirm the presence of the investigated compounds in positive samples, thus demonstrating that the DESI-HRMS method can represent a suitable tool for high throughput analyses.

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The future perspectives of mass spectrometry: an ever-wider horizon with use in clinical emergencies of toxicological interest.

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Keywords: *5F-APINACA, girl, intoxication*

Mass spectrometry is proving increasingly indispensable in clinical diagnosis by coupling speed of analysis and specificity in recognition. The close cooperation between the clinician and the laboratory technician provides useful data to direct the diagnosis and support the adequate post-diagnostic therapeutic treatment. By way of example, we highlight the multiplicity of substances for which it's possible to acquire a diagnostic address that may prove decisive and considerable in clinical performance. Among the cases that have come to my observation I mention the case of a 14 years old girl who had severe miosis and severe tachycardia with prevalence of reported hallucinations: she saw her long feet with a state of agitation and general illness. The examination of a residual sample taken an hour earlier, consisting of plant material of cannabis type, appeared to contain delta-9-tetrahydrocannabinol, JWH-018 and 5F-APINACA (5-fluoro-AKB48). The 5F-APINACA, already banned in some nations at the time, was still legal in Italy and there was no possibility of having the reference standard. For this reason, the identification was carried out with specialized reference sites (Cayman chemicals). Mass spectrometry also allowed a rapid diagnosis in an 84 years old woman who arrived at ER in a state of unconsciousness, accompanied by her cohabiting son. The slight reaction to flumazenil led to search for possible substances of benzodiazepines type, that were found as alprazolam, in cappelletti/tortellini that were handcrafted by the daughter-in-law that the lady had eaten.

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Synthetic isoflavones and doping: influence on the urinary steroid profile studied by gas chromatography – mass spectrometry

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Keywords: *Synthetic Isoflavones, Athlete Biological Passport, GC-MS, GC-Q-TOF.*

Flavonoids are a class of secondary plant characterized by 2-phenyl-1,4-benzopyrone basic structure. They occur in common human diet and have been used as drug or food supplement. The major activities of flavonoids are anti-oxidants, antiestrogenic and androgen promoters. In addition, several flavonoids are modulator of expression and activities of specific cythocrome P450 genes and/or proteins. Isoflavones are a group of flavonoids classified as "phytoestrogens" due to a weak estrogenic activities. Recently a renewed interest has been paid to the anabolic activity of isoflavones because they are potentially abused, as concomitant drug, during recovering period after the administration of anabolic steroids to increase the natural production of luteinizing hormone (LH) and consequently the synthesis of natural androgens [1-2].

In this work we have investigated the influence of two synthetic isoflavones, methoxyisoflavone and ipriflavone, on the urinary concentrations of steroid of doping relevance (testosterone (T), epitestosterone (E), androsterone (A), etiocholanolone (Etio), 5α-androstane-3α,17β-diol (5αAdiol), 5β-androstane-3α,17β-diol (5βAdiol) and the ratios T/E, A/T, A/Etio, 5αAdiol/5βAdiol, 5αAdiol/E) specifically considered in the framework of the Steroidal Module of the Athlete Biological Passport (ABP) [3] and their in vivo phase I metabolism.

Our results indicated that the administration of the selected synthetic isoflavones cause an alteration of the urinary concentrations of the investigated steroids and an increase in data dispersion that make more difficult the interpretation of the "longitudinal steroid profile" of the athletes. Beyond this, methoxyisoflavone and ipriflavone are extensively biotransformed in vivo following oral administration, with the production at least of ten (methoxyisoflavone) and eleven (ipriflavone) metabolites, that can be used as markers of the intake of these compounds.

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A Novel Approach for the Determination of OGSR by means UHPLC-HESI-HRMS

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Keywords: *OGSR, H-ESI, UHPLC-HRMS*

In recent years, due to the increasing use of ammunition without lead and heavy metals, forensic science has shown a growing interest in the detection of organic gunshot residues (OGSR). Despite GC-MS is still considered the golden standard [1], many LC-MS methods for the detection of OGSR can be found in the scientific literature. There are various references about APCI source operating in negative mode [2] which allow a soft ionization of the analytes and different methods for sample pretreatment, but, to date, there are few references to the use of the Electrospray source (ESI) [3]. Recently, it has been observed an increasing use of mass spectrometry as detection technique. Very common in the literature is the use of SIM (Single Ion Monitoring) as a method of acquisition [1]. To our knowledge, there are rare papers reporting the determination of both OGSR and stabilizers in a single chromatographic run.

Now, thanks to the coupling of high-performance liquid chromatography with high resolution mass spectrometry (UHPLC-HRMS) it is possible to achieve an innovative instrumental method that allows the simultaneous analysis of the most common OGSR and the three most used stabilizers (DPA, EC and MC). The 12 compounds were chromatographically separated by means of a Kinetex-C18 column. A chlorinated compound was added to the mobile phases, allowing to reveal the adducts with the chlorine formed directly in the chromatographic column. The ion source used is in this work is an H-ESI operating in positive and negative combined mode to allow the best conditions for each of the analytes. The revelation was conducted with a high-resolution mass spectrometer with Orbitrap technology that allowed us to work simultaneously in SIM and Full-dds so as to be able to detect both the parent molecules with and without the adduct with chlorine, and also the most intense fragments of the more stable compounds.

We are applying this method to the determination of OGSR and stabilizers both on forensic samples collected in crime scenes and oral fluids. In the latter case, different tests were carried out on habitual shooters and no-shooters, with the aim of obtaining blanks.

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New Psychoactive Substances: metabolite identification by means complementary approach

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Keywords: *NPS, Metabolism, UHPLC-HRMS*

In the field of the recognition of psychoactive substances, the knowledge of drugs' metabolic pathway is becoming increasingly important, with increasingly sensitive and accurate analytical techniques. In fact, our organism tends to apply substantial changes to the molecules taken in order to inactivate them and simultaneously expel them from the body. In the case of new psychoactive substances (NPS) this is even more difficult, given the lack knowledge on the pharmacokinetic appearance of these substances. The NPS abuse is associated with dissociate mental states and psychedelic sensations; because of the lack of legal restrictions these drugs are easily available. The difficulty in detecting the parent compound in urine, highlight the importance of metabolite identification for developing analytical methods for clinical and forensic investigations [1]. Having said that, this work aims to build a metabolic profile of NPS using the combination of three different types of approach: *in silico*, *in vitro*, and *in vivo*. In particular, the *in silico* experiment will allow to obtain preliminary data on the metabolism of these substances, using metabolic predicting programs such as MetaPrint2D (Cambridge Institute). The second phase concerns the incubation of substances using hepatocytes; this allowed us to obtain predominantly Phase I metabolites of the test substances, identifying them through the LC-HRMS analysis. Once identified, these metabolites were searched in test animals, which were treated with the substances to get a more complete picture on the phase II metabolism. Our research group has already successfully identified the metabolites of a new psychoactive substance known as MT-45 [2], and we are working to characterize two other psychoactive substances such as, Bromo-dragonfly [3] and Modafiendz, respectively a hallucinogen and a stimulator of cognitive activities.

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Case Report

Illicit administration of estradiol in cattle: case report

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Case context:

Endogenous steroids can be naturally present in body cattle fluids, but also illegally administered as growth promoters.

In 2014, a police investigation was performed in Piedmont Region. During the investigation, an undeclared animal treatment was observed by hidden cameras. Next day, anonymous liquids were impounded and serum of ten animals was collected and transferred to our laboratory.

Case resolution:

A rapid LC-MS/MS procedure was developed for simultaneous quantitative determination of progesterone, testosterone and 17β-estradiol at very low concentration levels in bovine serum. In 9 serum samples 17β-estradiol was found at concentration higher than Maximum limit. In all anonymous liquids the prodrug 17β-estradiol benzoate was found at 14 mg/ml.

Conclusions and/or perspectives:

The illicit treatment was found thanks to a collaboration between Police and the laboratory. Just one serum out of ten tested was found compliant. The practice of keeping one untreated animal in the same box with treated calves is typical in cattle breeding: it suggests the corruptive intent of the breeder to focus unfair veterinary controls on the untreated animal only.

USE OF SPME COUPLED WITH MASS SPECTROMETRY TO ASSESS THE USE OF SELF-DEFENCE PEPPER SPRAY

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Case context:

The use of self defence spray has recently been prohibited in mass events. Our goal was to understand if evidence of self defence pepper spray being sprayed could be found on physical evidence several months after the event.

Case resolution:

We tested several pieces of evidence where some parts were cut and put in an alcoholic solution. The SPME was put in contact with the solution so it absorbed the traces, and further analyzed with GC-MS. The method, previously tried on a test cloth, was able to find microtraces of capsaicin and dihydrocapsaicin on pieces of evidence.

Conclusions and/or perspectives:

Self defence spray in a crowd creates panic and confusion, with the result of a human wave that could lead to a deadly stampede. This method could be used to test evidence even after several months and help the public prosecutor to understand the origin of the event.

Investigations and analysis of cocaine from clandestine "laboratory"

Guido Persico, Maria Maglieri

Interregional Forensic Science Police Office of Naples

Case context:
A clandestine "laboratory" of cocaine was discovered by anti-drug team of Naples police headquarters in a city near Naples. On site inspection we found out that it was an apartment where two people (one of whom called "the chemist" was from South America) used to extract cocaine from various clothes. The cocaine was adsorbed on the clothes from South America.

Case resolution:
On crime scene, we carried out a color test to choose and seize the evidences to be submitted following further investigations. In the laboratory, we extracted cocaine from clothing, which was analyzed in GC-MS and GC-FID

Conclusions and/or perspectives:
In this report, we show the activity of the scientific police chemists that is not limited to laboratory tests but provides constant operational support to traditional surveys. The integration of skills and the exchange of information between the subjects involved in the criminal investigations are the assumption of new future investigative successes.

White Spirit, Black Widow (in the land of Red Chicory)

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Case context:

In 2010 I had a request for chemical investigations for the murder of a man: to detect fingerprints and analyse some chemicals. The suspect was the widow, with the help of three men. The items to analyse were collected at the crime scene and at the houses of suspects, notably a T-shirt, with a whiff of oil, found in the mouth of the dead. It was possible to link this evidence with one suspect?

Case resolution:

I analyzed with GC/MS, sampling with SPME, chemical adsorbed in the T-shirt, after sampling the blood traces for DNA typing, to compare it with seized chemicals. The issues to face: 1) a proper collection of evidence during the crime scene, 2) the sensitivity of analytical method, 3) the capability of linking the common chemical detected in the crime scene to the suspect's one.

Conclusions and/or perspectives:

The method was useful to identify, using ASTM E 1618, the chemical used in the murder: white spirit. Furthermore, it was possibile to claim the chemical detected in the murder weapon was more consistent with the white spirit seized to the suspected man, than some random "white spirit" standards. The challenge, in these cases, is to quantify the consistence of a product comparison.

Indoor Illegal Grow Marijuana Site, With Low THC Levels

Antonietta Lombardozzi[1], Morela Strano[1]

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Case context:
In the last few years it has been more and more frequent the seizure of marijuana grow sites. This case is the first big illegal seizure in Piedmont, of marijuana with low content of THC, analyzed by the Scientific Police Service of Turin.

Case resolution:
Our office intervention began on the site from the on-the-spot investigation together with the office that seized the site, to collecting evidence and samples to be analyzed. The analyses showed that the plants, even the ripe ones, had very low levels of THC.

Conclusions and/or perspectives:
The lack of documents to assess the legal status of the growing site and the low content of THC strongly indicates that the crop would have been sold as “marijuana for collection” in the cannabis shops. This is a trend that is increasing and poses a series of questions, from the legal-forensic perspective to the consumer safety.

Trace Evidence Analysis: Forensic examination of fibres

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Case context:

Forensic trace analysis can be a good source of evidence in all those cases in which neither biological samples nor fingerprints are found. In the present case, with regard to a fatal road accident between two motorcycles, the public prosecutor requested a comparison between some fibres found on the motorcycle fairing of *motorcycle 1* with those originating from the trousers worn by the victim, in order to ascertain the dynamics of the event.

Case resolution:

The traces found on the scene consisted of some blue and some white threads picked up by the *motorcycle 1*, some other yarns taken from *motorcycle 2* (driven by the victim) and the clothes of the victim, as the reference samples to compare. In order to answer the question, all the evidence were analysed by non-destructive analysis such as microscopy, infrared spectroscopy (FT-IR), and UV-Vis microspectrophotometry.

Conclusions and/or perspectives:

As a result of the technical examination, it was possible to conclude that the blue yarns from *motorcycle 1* were compatible, due to their morphology, chemical nature and chromatic tonality, with both the blue yarns sampled by the jeans of the victim and with those taken from the *motorcycle 2*. Recent developments in the use of innovative microscopic techniques are also reported.

Poster Presentation

Poster Presentation List

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PO06	**SPE-UPLC–ESI-MS/MS APPLICATION TO DETECTION OF CANNABIS SECONDARY METABOLITES IN WASTEWATER AND RIVER WATER** *S. Milan, A. Guimarães, L. Milella, F. Lelario, G. Bianco, L. Foti, S.A. Bufo, L. Scrano*

X-RAY POWDER DIFFRACTION FOR CHARACTERIZATION OF RAW MATERIALS IN BANKNOTES

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Keywords: X-ray Powder Diffraction, banknotes characterization, not-destructive analysis

We report about the X-ray powder diffraction (XRPD) characterization of crystalline materials used to produce genuine and counterfeit banknotes, performed with a single crystal diffractometer that permits fast and not-destructive measurements in different 0.5 mm sized areas. 20-euro denomination genuine banknotes were analyzed and results were compared with counterfeit banknotes.

The analysis shows that the papers used to print real banknotes are composed, as expected, of cotton based cellulose and titanium dioxide as crystalline additive, but different polymorphs of TiO_2 for different emission countries are evidenced. The counterfeit banknotes are composed of cellulose based on wood pulp, moreover an unexpected significant quantity of TiO_2 was found mixed with calcite, indicating that the paper employed by forgers is not simply a common low-cost type. The crystalline index and intensity ratios between the peaks attributable to cellulose and fillers can provide additional information to trace back paper suppliers for forensic purposes.

Veterinary Forensic Toxicology: confirmatory analysis by Gas Chromatography Mass Spectrometry (GC-MS)

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Keywords: *animal poisoning, toxicology, veterinary toxicology, GC-MS*

Animal poisoning is becoming a widespread phenomenon that has been extensively reported worldwide: animals are exposed to toxic agents in their environment both accidentally or intentionally, when some pesticides may be voluntarily distributed by humans (malicious poisoning) [1]. This phenomenon is also a real public health concern due to the high risk that children could come into contact with poisoned baits for playing or curiosity. Moreover, it is important not to underestimate the possibility that, due to cascade poisoning, toxicants can enter the food chain or spread into water.

In order to regulate the whole matter and to break the use of baits and bites containing toxic or noxious substances, starting from 2008, the Italian Ministry of Health issued some Ordinances regarding the "Rules on the Prohibition of Use and Detention of baits and or poisoned bites [2]. The main target was the control of poisoning episodes; then subsequent improvements were introduced to align our law with the EU Regulation 528/2012 [3] on the exchange and use of biocide products [4]. Despite this ban, the use of poisoned baits is still widespread in Italy as well as in the rest of the world. In most cases, exposure to pesticides is the primary cause of poisoning of domestic and stray animals in Italy, followed by molluscicides and rodenticides [5].

Within this framework, the Istituto Zooprofilattico Sperimentale del Mezzogiorno (IZSM) supports the investigation activities of the Public Organizations, such has the Italian Judiciary, the Municipal Police, the Carabinieri Corps, the Local Health Authority by the toxicological analysis on baits and biological matrices (liver, stomach and gastric contents) which are sent to their laboratories to confirm the poisoning suspects.

The toxicological Unit of IZSM uses gas chromatography mass spectrometry (GC-MS) to perform broad toxicological screening as well as routine confirmation for presumed positives in chemistry-based testing. GC-MS methods that include general solid–liquid extraction followed by a purification step have been developed and optimized to determine the presence of toxic substances commonly used in animal poisoning.

Chromatographic analysis was performed on a commercial Trace GC Ultra system (Thermo Fisher Scientific Inc., USA) coupled with a DSQ mass spectrometer and Xcalibur software (both Thermo Fisher Scientific Inc., USA) for data handling.

The GC system was equipped with an Elite 5MS column (PerkinElmer Inc., USA) (30 m × 0.25 mm) operating in electron impact mode (EI) at 70eV energy, source temperature at 280°C and transfer line at 250°C. The flow rate of helium gas was 1 mL/min, and the injection volume was 1.0 μL.

The GC-MS analysis was in Full Scan mode for the detection of pesticides, in order to monitor ions of charge ratio (abbreviated m/z) within a mass scan range between 50-500 m/z. This system allows us to detect compound fragments within that range over a set time period. Electron impact (EI) spectra of the unknown analytes are compared with NIST (National Institute for Standard and Technology) computer database, containing mass-spectra of many different compounds. A GC-MS method in SIM mode is used for detection of methaldehyde

setting the instrument to detect only masses of interest, being specific for the analyte of interest.
The results allowed us to detect several cases of violative samples in the Campania and Calabria regions. Suspected poisonings have been confirmed both in baits and biotoxicological matrices both due to the presence of many pesticides (such as endosulfan, phorate, parathion, methomyl, chlorpyriphos, omethoathe, dimethoathe, methamidophos, methiocarb) and of the molluscicide metaldehyde [6-7].
Moreover, through the use of geo-referencing systems it had been possible to identify the areas where there is a greater use of some substances, notifying it to the competent authorities in order to contribute to ensure the Public Security.

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Detection, Identification and Monitoring of chemical warfare agents by mass spectroscopy: a comparison between on-field and in-lab approach

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Keywords: *Chemical Warfare Agents; on-field detectors; Detection, Identification and Monitoring*

The use of hazardous chemical warfare agents, CWAs, in criminal acts or in warfare poses a non-negligible threat to the life of professionals and first responders (fire-fighters, police forces, military, emergency health service, etc.) involved in civil protection and emergency management actions [1]. Pre-hazard precautions as well as detection, identification and monitoring measures are the best options to prevent exposure and any form of contact between the toxic agent and the operator. In addition, not only the recent alleged use of CWA in the war scenario of the Middle East and the growing threat of dispersion of toxic compounds in terrorist attacks, but also the constant risk of accidental release of hazardous industrial materials in major incidents have stressed the key role of an early and effective on-site detection of potentially harmful agents [2,3].

In this aim, a prompt and advanced detection capability is crucial to get information about the potential risk, to identify the nature and the concentration of the contaminant, to provide a mapping of the hazardous areas and to define the protection and decontamination requirements.
Along with simple, although effective, manual and conventional detection systems, a broad variety of electronic and/or automatic hand-held detectors based on mass spectroscopy are available on the market for first responders who are expected to operate in a potentially contaminated and hazardous environment.

The poster will present a critical brief review of the most widely-known systems and apparatuses for the use on the field at an incident site, with a special emphasis on light-weight deployable gas-chromatograph mass-spectrometry (on-field GC-MS) instruments and ion mobility spectroscopy detectors (IMS; either with open loop or closed loop technology).

Advanced portable detector, based on IMS technology for the identification of toxic industrial materials and CWAs

Identification of an unknown sample by hand-held GC-MS apparatus in a potentially hot zone

Military-oriented reconnaissance armoured VBR NBC vehicle equipped with detection and identification instruments for chemical, biological and radiological hazardous agents

The particular aspects and differences between compact, portable instruments and conventional in-lab desktop ones will be highlighted. A comparison between civil-oriented detectors, for fire brigade, civil protection or emergency medical system units and military-oriented apparatuses will be outlined.

Some technical aspects of more complex integrated detection methods, such as the reconnaissance VBR NBC vehicle, in use by the Italian Armed Forces, will be treated as well.

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Foodborne intoxication by Bacillus cereus: an integrated protocol of analysis

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Keywords: *Cereulide, food, LC-MS/MS*

Background

Over 800 foodborne outbreaks caused by bacterial toxins take place in Europe every year (1). Some strain of Bacillus cereus that express *ces* gene are known to produce a peptide named cereulide, able to cause nausea and vomiting few hours after the ingestion of contaminated food (2). The toxin is a cyclic polypeptide stables at high temperature and acid pH; nowadays the minimun cereulide toxic dose is known to be 5 µg/kg.

The detection of toxigenic strains and the quantification of the toxin in food are concerns that official laboratory must deal with foodborne outbreaks management. The aim of the study was to validate and end-point PCR to detect *ces* gene encoding the cereulide produced by B. cereus group and to evaluate the performances of a LC-MS\MS method for cereulide quantitation.

Materials and methods

Detection of ces gene by duplex PCR end-point.

The DNA was extracted at 95°C and amplified. A total volume of 25 ul PCR mix was prepared, comprising 0.2 mM dNTPs, 2.5 mM $MgCl_2$, Buffer and $MgCl_2$ 1x, 0.6 µM e 1 µM primers, 1 UI HotStart Taq (Qiagen) (3,4). The primer sequences are shown in table 1. The strain B. cereus NCTC 11143 was used as positive control. LOD, sensitivity, specificity and selectivity were tested on a number of samples able to obtain a 95% confidence level.

Detection of cereulide by LC-MS/MS.

The LC-MS/MS quantitative method was developed according to ISO 18465:2017, but some changes were applied such as a double defatting step with n-eptan and filtration on 0.2 µm polypropylene filter. Chromatographic separation was achieved in X-Select HSS T3 column (150 x 3 mm, 2.5 µm) from Waters. Mobile phase [1] was 0.1% formic acid in methanol and Mobile phase [2] was 0.1% formic acid in aqueous ammonium formate 10 mM.

Mass spectrometry was performed in a triple quadrupole equipped with electrospray ionization source working in positive mode (ESI+) and the acquisition was performed by MRM mode. The precursor ion monitored was $[M+NH4]^+$ (m/z = 1170).

During the validation study, linearity, limit of quantitation, precision and recovery were determined. Moreover, the selectivity was assessed on several food matrixes containing pasta, rice, milk, cheese, potatoes, fish and meat.

Results and discussion

The duplex PCR end-point has shown a high sensitivity, specificity and selectivity for each matrix tested. The LOD resulted 10 UFC/25 g or mL for dairy products and 100 UFC/25 g or mL for the other matrixes.

The LC-MS/MS method was linear in the range 1÷100 µg/kg and the average CV% was < 2.5%. The method was not affected by matrix effect, thank to the use of SIDA approach with cereulide-C13 as internal standard.

The analytical protocol proposed in this study based on PCR and LC-MS\MS analyses showed high performances to be used in official laboratory during the investigations of suspected foodborne outbreaks caused by cereulide.

Project founded by Ministero della Salute (IZS PLV 09/16 RC)

Table 1. Primer gene *ces*

Primer CER1 – gene *ces* (2)	5' – ATCATAAAGGTGCGAACAAGA – 3'	188 pb
Primer EMT1 – gene *ces* (2)	5' – AAGATCAACCGAATGCAACTG – 3'	
Primer BCFW1 – gene *gyrB* (3)	5' – GTTTCTGGTGGTTTACATGG – 3'	352 pb
Primer BCRW1 – gene *gyrB* (3)	5' – CAACGTATGATTTAATTCCACC – 3'	

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Near-IR light emitting and absorbing materials for optoelectronics and trace analysis applied at interfaces and complex media

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Keywords: *OLED degradation – Differential photoluminescence – LC-(ESI)MS*

Organic electronic technologies, which include light emitting diodes, field effect transistors and organic photovoltaics, are vibrant research sectors from both the academic and industrial standpoint. Among optoelectronic devices, organic light emitting diodes (OLEDs) are considered the new reference technology suited for display and lighting applications thanks to their intrinsic low driving potentials (2-5 V) and broad color tunability. Although this technology has already been introduced in the market[1], two big challenges still remain to be solved: i) the instability and degradation of blue-emissive organic materials and ii) the lack of efficient emitters to extend the OLED *applications* into the nearIR region (λ_{NIR} > 700 nm) for safety, automotive, surveillance and phototherapy.

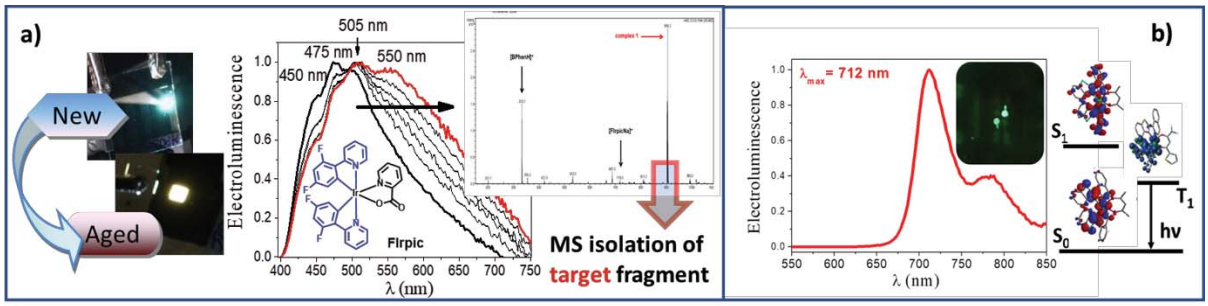

Figure 1. (a) "Aging" effects on working *blue*-OLED: perceivable color alteration, electroluminescence spectral evolution, and chemical structures of FIrpic). (b) NIR emissive $Ir(iqbt)_2dpm$ complex and its OLED application under IR night vision camera.

In the former case, the study of the degradation pathway leading to OLED failure is essential to meet the device lifetime and efficiency requirements[2]. Our approach combines differential-photoluminescence and thermal studies, device fabrication and LC-MS trace-chemical analysis aided by thorough theoretical investigation. We were able, applying HPLC-MS techniques with the isolation of the proper molecular ion to indisputably prove the degradation pathways as the result of a designed trapping event; in addition we define the boundary conditions for the suppression of degradations in a model blue OLED based on the archetypal FIrpic complex (Fig. 1a) [3]. In the NIR field, we undertook a rational structure-property strategy by the molecular engineering of Ir(III) emitters with *iso*quinoline-benzothiophene ligand (Fig. 1b) and we identified $Ir(iqbt)_2dpm$ as the leading system with

high NIR quantum efficiency of 16% and short radiative lifetime. Phosphorescent OLEDs were fabricated by solution processes and vacuum thermal evaporation showing state-of-the-art efficiencies exceeding 3% (Fig. 1b) [4].

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SPE-UPLC–ESI-MS/MS APPLICATION TO DETECTION OF CANNABIS SECONDARY METABOLITES IN WASTEWATER AND RIVER WATER

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Keywords: SPE-UPLC–ESI-MS/MS, *Cannabis Sativa* L., Wastewater

INTRODUCTION

Many environmental emerging organic contaminants, as pharmaceutical products and illicit drugs (IDs), are present in surface waters and in wastewater [1]. Drugs obtained by *Cannabis* are among them due to the large diffusion and accessibility of such substances, experienced by ¼ of European citizens during their lifetime [2]. Hemp and marijuana are two different types of Cannabis plants. Together with many other constituents, marijuana-type plants present high cannabinoids and Δ9-Tetrahydrocannabinol (THC) content, while hemp-types show significantly lower THC content (i.e. < 0,2%, w/w). Secondary wastewater effluent (SWWE) from a waste water treatment plant (WWTP) and water from the Febros River in the Porto urban area (Fig.1) were collected to investigate the occurrence of cannabinoids with the aim to confirm the need of tertiary treatment of waters to removal these kinds of contaminants.

Figure 1: Sampling site: (**Left**) WWTP in Vila Nova de Gaia, Porto urban area; (**Right**) Febros River [**3**], northern Portugal.

MATERIAL AND METHODS

All standards were purchased from Sigma Aldrich (Germany).
The experimental plan was:
-lab pre-experience spiking Milli-Q water with standard solutions to perform and validate robust analytical methods;
- water real samples collecting from SWWE and from the Febros River and their analysis.
Instrumentation : water ACQUITY UPLC® system (Waters, UK) with heated module interfaced to a triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source (TQD, Waters, UK).
Three different sorbent types (Oasis HLB, Oasis MCX and Oasis MAX) and quantities (1,3,6 mL) were evaluated using Milli-Q water spiked with the standard solutions. The nine considered analytes showed satisfactory absolute recoveries (65–130%) using Oasis MCX and Oasis HLB cartridges; the latter (6mL) was selected for the multiresidual method and for the semi-automated pre-concentration protocol.

RESULTS & DISCUSSION

Table 1 reports the cannabinoid MM, their ESI optimized via Intellistart™, LOD and LOQ, the Intermediate Precision (IP) for three replicates at 0.5 µg/L, and the repeatability (REP) as the RSD (%) of retention time and area for five repetitions at 0.125 µg/L on lab samples. Table 2 shows the quantization of cannabinoids in real samples. The analytes detected at very low concentration levels (below LOQ) were considered as positive findings when all the three transitions acquired were observed and at least one Q/q ratio was within the tolerance limits. The multiresidual method shows that these residues were not completely removed in the WWTP, suggesting that an advanced tertiary treatment could be necessary to increase the performance of the plant and achieve a complete disappearance of these recalcitrant substances.

Table 1. Molecular mass (MM), ionization (ESI) mode, working range (µg/L), determination coefficient (R^2), limit of quantification (LOQ), limit of detection (LOD), intermediate precision (IP) and repeatability (REP)

Compound	MM	ESI	Range (µg/L)	R^2	LOQ (S/N)=10	LOD (S/N)=3	IP (%)	REP(%)
CBDV	286,41	-p	0.050-0.500	0.9995	0.046	0.015	6.1	6.8
CBN	310,43	-p	0.050-0.500	0.9953	0.147	0.048	10.0	5.3
CBD	314,45	+p	0.050-0.500	0.9991	0.065	0.021	2.6	8.8
CBG	316,48	+p	0.050-0.500	0.9995	0.047	0.016	8.0	24.8
THV	286,41	-p	0.050-0.500	0.9990	0.070	0.023	8.2	7.2
CBDA	358,47	+p	0.050-0.500	0.9986	0.080	0.026	5.9	17.1
THC	314,45	+p	0.050-0.500	0.9991	0.063	0.021	5.8	21.8
THCA-A	358,47	-p	0.050-0.500	0.9980	0.098	0.032	4.9	11.2
THC-COOH	344,45	+p	0.050-0.15	0.9987	0.019	0.006	3.8	13.8

Table 2. Concentration (l/L), of the targeted cannabinoids and recoveries (Rec) with their respective % RSD, quantitation in effluent wastewater (WWE) samples from the WWTP located in Vila Nova de Gaia and in Febros River

	Febros River				WWE			
Compound	[µg/L]	RSD (%)	Rec (%)	Rec RSD (%)	[µg/L]	RSD (%)	Rec (%)	Rec RSD (%)
CBDV	<LOQ	-	101	2.5	<LOQ	-	96	9.4
CBN	0.079	3.0	91	1.1	0.076	4.8	92	2.2
CBD	<LOQ	-	100	13.7	0.096	4.3	133	1.8
CBG	<LOQ	-	73	8.5	<LOQ	-	64	15.0
THV	<LOQ	-	123	1.3	<LOQ	-	84	0.9
CBDA	0.070	7.9	110	1.2	0.065	0.0	125	3.2
THC	<LOQ	-	112	3.2	<LOQ	-	133	1.3
THCA-A	0.073	8.7	110	1.7	0.071	5.8	116	4.3
THC-COOH	<LOQ	-	102	6.3	<LOQ	-	71	9.8

.....Concluding

Results of this scientific work evidences as the mass spectrometry is very important for the detection at low concentration of the cannabis secondary metabolites (cannabinoids) in SWWE. Obviously, more comprehensive studies on the occurrence of this class of emerging contaminants (i.e. CBD, CBDA, CBN, THCA-A) in the aquatic environment are needed to better understand the usage of this plant metabolites (used as drug or pharmaceuticals). The most studied cannabinoid THC and its main human metabolite THC-COOH was not present at relevant concentrations in River and WWE samples as described in [4]. The secondary treatment applied in the WWTP seems not to be effectively and completely removing this class of compounds [5] as significant amounts are released into surface water. For this reason a water tertiary treatment could be necessary and suggested.

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