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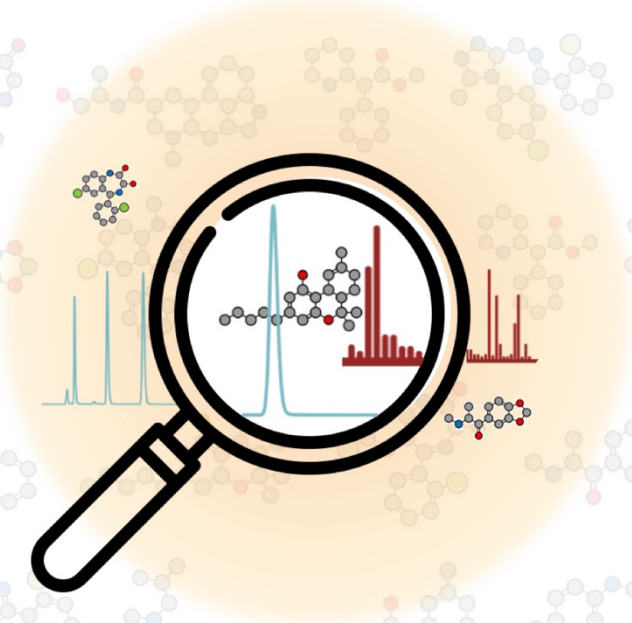
PTA Lab Pharmaco Toxicological Analysis Laboratory

IMaSS Italian Mass Spectrometry Society

IMaSS Drug Analysis Day

A day dedicated to pharmaceutical and pharmaco-toxicological analysis in biological and non-biological samples



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BOOK OF ABSTRACTS

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Welcome

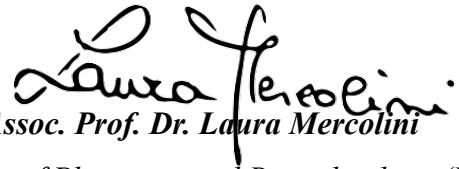
As the field of drug analysis continues to advance rapidly, it is essential for professionals, researchers and students alike to come together and exchange knowledge, ideas and insights.

This day serves as a remarkable platform where experts from various disciplines gather to discuss the latest advancements, breakthroughs, and challenges in drug analysis. Our esteemed speakers, renowned scientists, and industry leaders will provide in-depth presentations and engaging discussions on a wide range of topics. There will be the chance to connect with like-minded individuals, foster collaborations and explore innovative technologies and methodologies shaping the future of the field.

Join us at IMaSS Drug Analysis Day to gain valuable insights, share your expertise, and contribute to the advancement of this crucial scientific domain. Together, let us pave the way for safer, more efficient, and more reliable drug analysis methods that will positively impact society.

On behalf of the Organizing Committee, we look forward to welcoming you to this exceptional event and wish you a memorable and knowledge-filled experience.

The IMaSS Drug Analysis Day 2023 Congress Chair



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BEST POSTER AWARD

During the afternoon break (at 17:30), the Conference Hall will come to life with the award ceremony for the best poster presentation.

The prize is **a free one-year membership fee to IMaSS – Italian Mass Spectrometry Society**, awarded by the Society President, Dr. Andrea Armirotti, and presented by the Chair of the Award Commission, Dr. Andrea Raffaelli.

SCIENTIFIC PROGRAM

Morning Session

Chairs: Laura Mercolini, Andrea Armirotti

9:30 Laura Mercolini and Andrea Armirotti

Opening and introduction

10:00 Guido Pelletti (University of Bologna)

Application of mass spectrometry in post-mortem forensic toxicology

10:30 Giampietro Frison (AULSS 3, Venice)

LC-HRAM-Orbitrap-MS determination of ethyl glucuronide in blood, urine and hair samples

11:00 Alberto Salomone (University of Turin)

Mass Spectrometry-based approaches to investigate the prevalence of NPS and doping agents

11:30 Coffee break

12:00 Carlotta Stacchini (FMSI Anti-doping Lab)

Application of MS-based techniques for the analysis of novel proteins and peptides of anti-doping relevance

12:30 Federica Pellati (University of Modena and Reggio Emilia)

Chromatographic methods and biological assays to disclose the antiproliferative activity of cannabidiol against chronic myelogenous leukemia cancer cells

13:00 Federica Ianni (University of Perugia)

Characterization of (bio)active compounds in vegetable matrices supported by LC-HRMS/MS

13:30 Lunch break & Poster session

Afternoon Session

Chairs: Federica Pellati, Roberto Mandrioli

14:30 Michele Protti (University of Bologna)

Microsampling and mass spectrometry for patient-centric TDM studies

15:00 Luca Regazzoni (University of Milano)

MS studies on a new mode of action of carnosine

15:30 Coffee break & Best poster award

16:00 Alessandro Armandi (DaSP – Sciex channel partner)

Comprehensive characterization of protein therapeutics using electron activated dissociation (EAD)

16:30 Giulio Calza (Bruker)

MALDI-2: Regaining sensitivity in high spatial resolution Imaging for endogenous and xenobiotic compounds

17:00 Closing remarks

ORAL PRESENTATIONS

APPLICATION OF MASS SPETTROMETRY IN POST-MORTEM FORENSIC TOXICOLOGY. PITFALLS AND FUTURE CHALLENGES

Guido Pelletti[1,*]

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The role of forensic science can be defined as providing relevant opinions in a logical and correct way, to assist investigators and courts of law in answering questions. In all fields of forensics, it is essential for experts to know how to achieve reliable and reproducible results, but also to provide an appropriate interpretation. In post-mortem forensic toxicology, the introduction of mass spectrometry (MS) and high-resolution mass spectrometry (HRMS) has brought revolutionary changes. These advanced techniques, along with the use of targeted and untargeted approaches, have significantly enhanced the ability to identify substances that were previously undetectable, as well as complex drug combinations [1]. Pitfalls of MS analysis encompass challenges in sample preparation, potential matrix effects, interferences from endogenous compounds, and limited reference standards, all of which can compromise the accuracy and reliability of results. Forensic toxicology should also face the emerging trend of novel psychoactive substances (NPS) and the need for continuous updates in analytical methods to detect these ever-evolving compounds. Addressing the pitfalls and tackling future challenges will be essential to further advance and consolidate the role of mass spectrometry as an indispensable tool in forensic toxicology, contributing to the accurate determination of cause of death and ultimately supporting justice and public safety.

The routine and recent innovative applications of MS in the forensic toxicology laboratory in Bologna are presented and discussed in this context [2, 3]. These applications include the analysis of fly artifacts, as well as the analysis of gases and volatiles in cases involving suspected acute poisoning. These advanced techniques have shown great potential in identifying the cause and manner of death in forensic investigations.

References:

[1] Wille SMR, Desharnais B, Pichini S, Trana AD, Busardò FP, Wissenbach DK, Peters FT. *Curr Pharm Des.* 2022;28(15):1230-1244.

[2] Giorgetti A, Pelletti G, Fais P, Giovannini E, Barone R, Pelotti S, Pascali JP. *J Forensic Sci.* 2022 Mar;67(2):749-755.

[3] Giorgetti A, Pelletti G, Barone R, Garagnani M, Rossi F, Guadagnini G, Fais P, Pelotti S. *Forensic Sci Int.* 2020 Dec;317:110548.

LC-HRAM-ORBITRAP-MS DETERMINATION OF ETG IN BLOOD, URINE, AND HAIR SAMPLES AFTER DERIVATIZATION WITH EDC

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The LC-MS determination of Ethyl Glucuronide (EtG) in biofluids may be challenging because of its problematic behaviour with RP LC columns and the need to adopt negative ESI conditions, due to its polar and acidic properties, and the absence of nitrogen atoms in the molecule. To overcome these limitations, we derivatized EtG and D_5-EtG with the carbodiimide EDC, structurally characterized their derivatives by LC-HRAM-Orbitrap-MS and detected EtG levels in blood, urine, and hair samples of teetotalers and drinkers.

MASS SPECTROMETRY-BASED APPROACHES TO INVESTIGATE THE PREVALENCE OF NEW PSYCHOACTIVE SUBSTANCES

Alberto Salomone[1]

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Forensic toxicology aims to apply newly developed methods to resolve complex intoxications or judicial cases. Quite often, only small quantities of sample (either a biological sample or a seized material) are available, in which the analyte is present at trace level. Furthermore, new and unknown compounds are continuously introduced onto the black market, introducing a risk of 'missed cases". In this scenario, innovation is highly needed, in terms of more sophisticated methods, improved access to alternative matrices, advancement of multivariate statistical and machine learning tools. Traditional forensic toxicology involves screening methods designed to detect the traditional drugs of abuse. In case of a non-negative screening result, the samples are re-analyzed with targeted confirmatory assay, usually based on tandem-mass spectrometry. With the explosion of new psychoactive substances (NPS) and novel synthetic opioids (NSO) in the past decade, it has become necessary to reevaluate traditional targeted strategies. One of the challenges for the analysis and identification of NPS/NSO in a rapidly changing and dynamic market is that analytical reference materials may often not be commercially available, or be extremely costly in terms of time, work and money for purchase. Therefore, it appears almost impossible for most laboratories to have up-to-date target methods, able to detect the hundreds of compounds. One possible solution may come from untargeted analysis by means of HRMS acquisition, either based on a retrospective data analysis of previously acquired data, or on untargeted analysis of the metabolome. Further serious challenges to detect the presence of NPS in biological matrices, especially urine, are posed by the extensive metabolic transformation that these substances undergo once introduced into the body. Furthermore, most drugs are eliminated from blood and urine within a few hours or days. Thus, only very recent use can be detected, preventing clear knowledge of the real consumption of these new drugs in the population. To circumvent these limits, the detection of the parent drugs in hair has been proposed, as well as the possible triangulation with other sources of information, such as wastewater analysis or epidemiology studies based on surveys. When the screened population and the target analytes are extended, the resulting picture becomes certainly more definite and understandable. This approach can be applied to several scenarios, for example to elucidate drug diffusion during the COVID-19 era, to point out the unaware use of NPS, or to investigate the adulteration of low-THC Cannabis with synthetic cannabinoids.

APPLICATION OF MS-BASED TECHNIQUE FOR THE ANALYSIS OF NOVEL PROTEINS AND PEPTIDES OF ANTIDOPING

Carlotta Stacchini[1*], Xavier de la Torre[1], Daniel Jardines[1], Francesco Botrè[1,2]

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The application of mass spectrometric techniques for analyzing novel proteins and peptides of antidoping relevance has gained significant attention in recent years. As the sports field continues to evolve, the need for accurate and sensitive methods to detect novel performance-enhancing substances becomes paramount. Protein-based substances are attractive alternatives for those athletes that seek to gain an unfair advantage, improving their sport performances. Therefore, peptides and proteins are present in the Prohibited List of the World Anti-Doping Agency, mainly in sections S2 (Peptide Hormones, Growth Factors, Related Substances, And Mimetics), S4 (Hormone And Metabolic Modulators), and S5 (Diuretics And Masking Agents). These molecules can activate or modulate biological processes, presenting the advantages of having a short half-life in the body and/or being identical or similar to its natural components, making their detection challenging for antidoping laboratories. Seizures by competent authorities demonstrate athletes' widespread use of these molecules. So, one of the primary purposes of the antidoping laboratories, in addition to the molecular characterization by mass spectrometry of new peptides and proteins circulating on the (black) market, is developing analytical methods for their detection in accredited biological matrices (urine and blood). Here we present the different approaches to detect the illicit intake of protein-based substances: (i) the development of direct methods that allow the unique identification of the substances, if the molecules are structurally different than the human endogenous components (e.g., IGF-1 analogs, synthetic insulins, BPC-157); (ii) the development of indirect strategies, such as the endocrinological module of the athlete's biological passport, if the proteins are structurally identical to their endogenous counterpart (e.g., hGH, IGF-1). Combining direct and indirect methods based on mass spectrometric techniques for detecting new doping substances represents the winning strategy in the fight against doping.

References

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CHEMICAL COMPOSITION, *IN VITRO* ANTIPROLIFERATIVE ACTIVITY OF NON-PSYCHOACTIVE *CANNABIS SATIVA* L. EXTRACTS AND PROTEOMIC IDENTIFICATION OF CANNABIDIOL INTERACTOME

Lorenzo Corsi[1], Sara Ceccacci[2], Clarissa Caroli[1,3], Lisa Anceschi[1,3], Virginia Brighenti[1], Matteo Mozzicafreddo[4], Maria Chiara Monti[2], Federica Pellati[1,*]

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Extracts from non-psychoactive *Cannabis sativa* L. (hemp) varieties were fully characterized in this work by means of ultra high-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS) and their cannabinoids were quantified using HPLC-UV. The antiproliferative activity of the extracts was assessed *in vitro* against a panel of human cancer cell lines. The chronic myelogenous leukemia K562cell line was the most sensitive one to a CBD-type hemp extract and, therefore, it was chosen to investigate the mechanism of cell death. The effect on cell cycle and cell death was analyzed by flow cytometry. Proteins related to apoptosis were studied by western blotting. The results indicated that the cannabidiol (CBD)-rich extract inhibited cell proliferation of K562 cell line in a dose-dependent manner and induced apoptosis via caspase 3 and 7 activation. A significant decrease in the mitochondrial membrane potential was detected, together with the release of cytochrome c in the cytosol. The main apoptotic markers were not involved in the mechanism of cell death. To shed light on CBD apoptotic mechanism of action, a functional proteomic study based on "Drug Affinity Responsive Target Stability" (DARTS) was performed to identify its interactome in K562 cells. DARTS results were validated by immunoblotting and targeted-Limited Proteolysis-Multiple Reaction Monitoring Mass spectrometry (t-LiP-MRM). The proteomic platform revealed the 116 kDa U5 small nuclear ribonucleoprotein component (EFTUD2), a highly conserved spliceosomal GTPase, as a CBD novel cellular target. It was found that the peptides protected by CBD, i.e. S[183-194]K and I[326-341]K, belong to the GTP binding domain of EFTUD2. EFTUD2 is described in the literature as a protein that, when de-regulated, promotes the survival of several cancer cells through the activation of STAT3. Finally, CBD apoptotic effects on K562 cell lines may be due to its ability to affect the GTPase activity of EFTUD2, affecting apoptosis via STAT3.

CHARACTERIZATION OF (BIO)ACTIVE COMPOUNDS IN VEGETABLE MATRICES SUPPORTED BY LC-HRMS/MS

Federica Ianni*, Lina Cossignani, Francesca Blasi, Roccaldo Sardella

Department of Pharmaceutical Sciences, University of Perugia, via Fabretti, Perugia, 06126 (Italy)

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Agri-food wastes and by-products are complex mixtures containing precious (bio)actives, the recovery of which represents an important and continuously growing economic and environmental challenge. In this framework, chromatographic techniques are the elective choice for the determination of ingredients from food waste in line with the new trends in the food, cosmetic and pharmaceutical fields. The high structural variability of naturally occurring food compounds, such as polyphenols and carotenoids, together with the limited availability of commercial standards, complicates their identification. In addition, the chemical transformation they may undergo during food processing requires continuous monitoring to ascertain their integrity or identify potential side-products that could deeply affect the biological properties. For this reason, the hyphenation of LC to MS offers several benefits, allowing unique identification of (bio)active compounds in complex matrices (e.g. when peak overlap is a pitfall) or transformation products. Moreover, LC-MS systems represent an unavoidable final analytical step to confirm the identity of molecules previously analysed through most widely used UV or DAD detectors. This is the leitmotif of the case studies presented in this work (Fig. 1), where LC-HRMS/MS investigations were carried out in support of HPLC-DAD analyses to allow the characterization of carotenoids in pumpkin extracts and of the phenolic profile in potato, grape- and apple-pomace by-products [1,2]. Additionally, thorough HPLC-HRMS/MS investigations were performed to confirm the identity of chlorogenic acid isomers and enable the structural elucidation of their transformation products after microwave-assisted extraction [3].

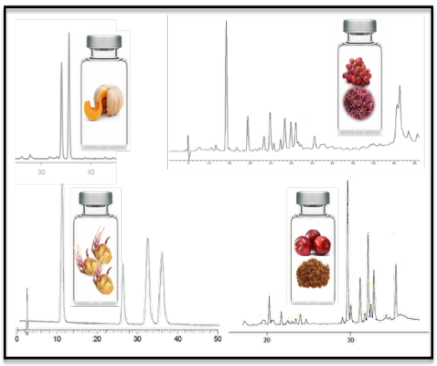

Fig. 1: Case studies of this work.

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[1] N. Pinna, F. Ianni, F. Blasi, A. Stefani, M. Codini, S. Sabatini, A. Schoubben, L. Cossignani, *Molecules, 27 (2022) 8240.*

[2] L. Mangiapelo, F. Blasi, F. Ianni, C. Barola, R. Galarini, G. Abualzulof, R. Sardella, C. Volpi, L. Cossignani, *Antioxidants, 12 (2023) 348.*

[3] F. Ianni, C. Barola, F. Blasi, S. Moretti, R. Galarini, L. Cossignani., *Food Chem., 374 (2022) 131820.*

MICROSAMPLING AND MASS SPECTROMETRY FOR PATIENT-CENTRIC TDM STUDIES

Michele Protti[1*], Sarah Palano[1], Mohammadreza Mahdavijalal[1], Roberto Mandrioli[2], James Rudge[3], Anna Rita Atti[4], Diana De Ronchi[4], Andrea Armirotti[5], Andrea Cavalli[1], Laura Mercolini[1]

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Liquid chromatography coupled to MS is currently considered the golden standard in most bioanalytical applications, including pharmaceutical and clinical ones, and it is ideally suited for hyphenation with miniaturised sampling and pretreatment procedures, which deal with sample volumes approximately ranging from a few to tens of microliters. Microsampling, and dried microsampling in particular, is advantageous for most bioanalytical applications, allowing minimal invasiveness to the subject and collection when sample availability is very limited in volume, also allowing feasible and practical shipping and storage without the need for refrigeration or freezing, also enabling patient home-sampling and feasible, streamlined protocols [1]. An original LC-MS/MS method coupled to microsampling will be presented, dealing with the analysis of central nervous system (CNS) drugs and metabolites for therapeutic drug monitoring (TDM) purposes, namely new-generation antidepressant agents (ADAs). Satisfactory method validation and application results have been obtained by designing an original workflow based on volumetric absorptive microsampling (VAMS) [2], also by comparing datasets obtained from gold standard methodologies based on classic fluid plasma analysis from patients under treatment with ADAs. Considering the promising results obtained, microsampling could become a relevant ally of mass spectrometry in bioanalytical applications for CNS drugs TDM in the perspective of patient-centric clinical approaches and personalised medicine.

References:

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MS STUDIES ON A NEW MODE OF ACTION OF CARNOSINE

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Carnosine (i.e., β-alanyl-L-histidine) is a naturally occurring dipeptide that gave positive outcomes in several pharmacological tests performed on animal models of human disease [1]. The mode of action of such a peptide has been reported to be a prevention of oxidative modification of proteins and DNA, by a combination of direct and indirect mechanisms [2]. In fact, carnosine can directly bind oxidants and electrophiles or indirectly stimulate cell defense by activating specific pathways (e.g., Nrf2).

The data herein reported complement carnosine mode of action with another important feature. Specifically, mass spectrometric studies allowed to collect evidence of the reversible nature of protein modifications as induced by electrophiles such as 4-hydroxynonenal (HNE). Importantly, carnosine and other histidine dipeptides were able to accelerate such a process (i.e., decarbonylation) in a dose dependent manner.

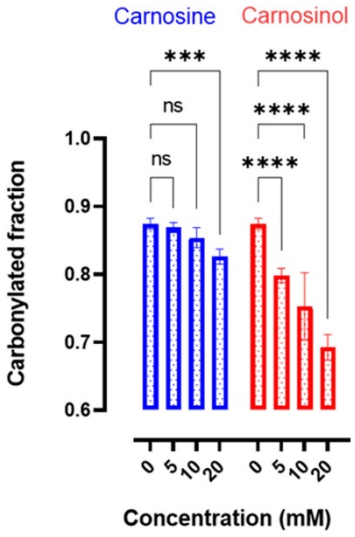

Fig. 1: protein decarbonylation as induced by carnosine and carnosinol

HNE modification of proteins has been described as an irreversible process involved in several human diseases [3]. The evidences that HNE protein damage can be repaired, and that such a process can be promoted by carnosine are therefore important not only for expanding the knowledge around the modes of action of carnosine, but also to understand the mechanisms of HNE trafficking into the cells..

References:

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COMPREHENSIVE CHARACTERIZATION OF PROTEIN THERAPEUTICS USING ELECTRON ACTIVATED DISSOCIATION (EAD)

Cermenati G.[1], Scurati S.[1], Liu, H.[2], Fiorina S.[1], Armandi A.[1,*]

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The growth of protein therapeutics has largely been due to advances in recombinant DNA technology that have provided the means to produce protein therapeutics in the discovery of novel protein drugs. However, the characterization of these drugs brings with it numerous analytical challenges due to the structural complexity of these molecules but above all to the production complexity underlying these new therapies.

As one of the most highly utilized analytical techniques in pharmaceutical research and development, mass spectrometry (MS) has been widely used in the characterization of protein therapeutics because of its analytical sensitivity, selectivity, and specificity [1].

In this scenario, the search for new tools to better characterize therapeutic proteins is always current. For this purpose, the electron-activated dissociation (EAD) offered by the ZenoTOF 7600 system is a new powerful fragmentation tool (complementary to that classically used in ESI-LC-MS/MS systems) to ensure a better characterization of protein therapies [2]. Moreover, compared to traditional low-energy approaches, the Zeno Trap-enhanced EAD offers faster scan speed and higher sensitivity, making this analytical approach suitable for routine biopharmaceutical characterization.

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MALDI-2: REGAINING SENSITIVITY IN HIGH SPATIAL RESOLUTION IMAGING FOR ENDOGENOUS AND XENOBIOTIC COMPOUNDS

Giulio Calza[1*], Corinna Henkel[2], Annika Koch[2], Michael Becker[3], Tobias Orth[3], Jan Hendrik Kobarg[2], Shannon Cornett[4], Cristina Silvescu[4], Aiko Barsh[2], Michael Easterling[4]

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The combination of MALDI with laser-induced post-ionization (MALDI-2) has demonstrated significant enhancement of sensitivity in mass spectrometry imaging [1,2]. Fresh-frozen liver and kidney tissue sections were cut in 10 µm thickness and mounted on Bruker IntelliSlides. After drying, a 0.5 µl mixture of the following substances were spotted on top of the tissue: caffeine, chloroquine, rosuvastatin, BI-YYY, and reserpine. Each mixture was spotted in a dilution range from 1 µM to 100 µM (9 spots). The slides were then coated with 2,5-Dihydroxyacetophenone (DHAP) matrix (15 mg/mL in ACN:H_2O:MeOH, 8:1:1, v:v) using a TM-sprayer (HTX Technologies). MALDI Imaging data was collected on a timsTOF fleX with MALDI-2 technology. Mass spectra were acquired in positive ion mode, with a m/z range of 100-2000 and 50 µm pixel size. For comparative measurements, the MALDI-2 laser was turned on/off as needed. The same parameters were used for dosed tissue imaging of liver and kidney slices after oral administration with either 100 mg/kg chloroquine or 120 mg/kg of BI-YYY. Spotting experiment demonstrated that sensitivity for all five test compounds was significantly increased by MALDI-2. In particular, the peak intensity of the new drug compound, BI-YYY, was enhanced by a factor of 300 using MALDI-2. For the treated tissues the signal intensity for chloroquine in kidney was 6-fold greater using MALDI-2. For chloroquine in liver, results were similar where mean peak intensity reveal a 5-fold higher intensity boost from MALDI-2. Interestingly most chloroquine metabolites which generated only weak signals using traditional MALDI were visible in the MALDI-2 experiment, delivering new distribution information from previously undetected metabolites as well as providing lower limits of detection for target compounds, both vitally important to DMPK studies.

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POSTER CONTRIBUTIONS

UHPLC-HRMS GUIDED IDENTIFICATION OF HEMP PHENOLIC COMPOUNDS BIOACTIVE AGAINST COLORECTAL CANCER

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Colorectal cancer (CRC) is one of the most diagnosed cancers in high-income countries, and one of the main concerns is that it can easily develop multidrug resistance [1,2]. Polyphenols can be useful to overcome this issue, since they can act against cancer cells with several mechanisms of action [2]. Phenolics from non-psychoactive *Cannabis sativa* L. (hemp), in particular cannflavins, are known to possess several biological properties. However, their antiproliferative activity has not been deeply investigated [3]. In the light of this, the aim of this study was to obtain and characterize, by ultra-high performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS), a polyphenols-enriched fraction (PEF) from hemp inflorescences and to test its antiproliferative activity against CRC cell lines, together with pure compounds. The work started with the optimization of a new extraction method having a high selectivity for polyphenols, followed by preparative flash column chromatography under normal phase conditions. UHPLC-HRMS was applied to fully characterize PEF, while HPLC-UV was applied to quantify its main compounds. The antiproliferative activity of the PEF and pure compounds was assessed against Caco-2 and SW480 cell lines, after 24 and 48 h of treatment, providing promising IC_{50} values, in comparison with cisplatin. Based on these results, PEF represents an interesting product to be further investigated for its bioactivity.

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A GLIMPSE INTO HASHISH CHEMISTRY: COMPARISON OF LC-MS/MS AND ATR-FTIR FOR THE ANALYSIS OF THC AND THCA

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The properties of Cannabis and its use for medical and recreational purpose are well-documented, and Δ^9-tetrahydrocannabinol (THC) is known as the main psychoactive substance present in the plant [1]. The growing focus on the abuse potential of Cannabis has led to new requirements for more accurate and effective research on the matter. Most analysis techniques require time-consuming sample preparation protocols; thus, this study was focused on the development of a fast, direct method of analysis for THC and its carboxylic acid precursor, THCA in hashish. Attenuated total reflectance-Fourier's transform infrared spectroscopy (ATR-FTIR) was chosen due to its ease of use, solvent-free nature, and non-destructivity [2]; however, the IR spectral differences between the two analytes are quite small and hardly identifiable in a complex hashish sample. For this reason, a novel ATR-FTIR method had to be developed to simultaneously measure the levels of THC and THCA in hashish samples using a direct process. The method was then compared and cross-validated with an original, benchmark LC-MS/MS one already developed in-house. The accuracy range for THCA varied from -15.25% to 51.62%, with RSD of 1.57-3.01%; the limit of quantification (LOQ) was 19.10% w/w. Accuracy for THC ranged from -75.35% to 23.91%, with RSD of 0.23-0.59%; LOQ was 2.76% w/w. The data demonstrated that, compared to LC-MS/MS, ATR-FTIR had a tendency to overestimate the concentration of both analytes at lower concentrations and underestimate it at higher concentrations. Assays are in progress to increase accuracy homogeneity.

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ICP-MS MONITORING OF CADMIUM OCCURRENCE IN CHOCOLATE BARS TO ASSESS EXPOSURE IN POLES

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Cadmium is a heavy metal naturally occurring in its inorganic forms in the environment. It can be absorbed from the diet and accumulates in kidneys and liver, with a biological half-life of 10-35 years. Over time, its accumulation can create several health adverse effects including renal dysfunction and renal failure [1]. Since cadmium in chocolate derives only from the cocoa beans and not from processing contamination, its content in chocolate depends on the percentage, the quality and the origin of cocoa [2]; thus, a maximum allowed cadmium level in chocolate, ranging from 0.10 to 0.80 µg/g wet weight, was set by the European legislation [3]. The aim of this work is to define the occurrence of cadmium in different chocolate types on the Polish market by inductively-coupled plasma ionisation – mass spectrometric (ICP-MS) analysis and to estimate exposure in different Polish population groups. Several chocolate bars were subjected to microwave-assisted mineralization (1800 W, 210°C) and were analysed through single quadrupole ICP-MS. Kinetic energy discrimination module filled with helium was used and cadmium quantification was performed with external standard calibration. Results showed that cadmium levels in chocolate are in all cases below the cadmium limit set by European legislation [3] and that cadmium exposure in the Polish population does not exceed the tolerable weekly intake proposed by EFSA's scientific opinion [1].

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OPTIMIZING SORBENT CHOICE FOR THE APPLICATION OF LC-MS/MS TO THERAPEUTIC DRUG MONITORING OF ANTIPSYCHOTICS

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LC-MS/MS methods are the gold standard in drug analysis for the therapeutic drug monitoring (TDM) of patients, a form of precision medicine that includes the determination of drug levels in patient plasma to aid in assessing therapy efficacy and preventing side effects and drug toxicity. Despite the outstanding selectivity and sensitivity of LC-MS/MS, sample pre-treatment is still a key step in this kind of bioanalytical application, since the complex biological matrix can cause strong matrix effects, background noise and analytical interference [1]. Solid phase extraction (SPE) and related techniques are widely applied for this purpose; traditionally, silica-based C_{18} sorbents are the standard choice when dealing with lipophilic drugs, such as antipsychotics and other central nervous system (CNS) agents. Other attractive options are available, such as polymer-based hydrophilic-lipophilic balance (HLB) and lipophilic/ion exchange mixed-mode sorbents [2]. The multiple retention mechanisms afforded by these sorbents allow the user to carefully calibrate selectivity. In this study, C_{18}, HLB and mixed-mode sorbents and procedures were compared to find those most suitable for application to LC-MS/MS analytical workflows for the TDM of patients undergoing CNS drug therapies. A proof-of concept application to clozapine, the reference drug for antipsychotic therapy, has been tested with satisfactory results when using an SPE procedure based on the HLB sorbent. Sample purification was good in terms of both extraction efficiency (> 90.4%) and clean-up; coupling to LC-ESI-MS/MS with triple quadrupole detection produced analytical performance practically devoid of matrix effect.

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ANALYSIS OF OPIOIDS IN BIOLOGICAL MATRICES BY UHPLC-MS

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Opioids are a class of very powerful drugs used in the field of acute and chronic pain therapy. They have also a high potential for abuse and addiction and pose a high risk of toxic effects in overdose. For this reason, analytical techniques that allow the simultaneous analysis of multiple opioids are highly valued. LC-MS is the technique of choice for this kind of determination due to its outstanding selectivity; however, the existence of isobaric metabolites or analogues makes this task nontrivial and complete chromatographic separation becomes paramount to the applicability of the method [1]. In this study, an innovative analytical method has been developed, capable of separating and quantifying several opioids by UHPLC-MS. Fentanyl and metabolites, morphine, tapentadol and tramadol and metabolites can be determined using a C18 stationary phase and an acetonitrile/water mixture under composition gradient as the mobile phase; oxycodone and isobaric metabolites can also be baseline separated using a carefully optimised elution gradient, allowing their accurate determination despite the molar mass identity. The method is currently being validated for application to human plasma and whole blood microsamples collected through volumetric absorptive microsampling (VAMS) [2].

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DIRECT ANALYSIS OF COUNTERFEIT DRUGS WITH A NEW MINIATURE, FIELD DEPLOYABLE MASS SPECTROMETER

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Over-the-counter (OTC) medications, available without a prescription, encompass a wide range of products used to alleviate common ailments, including pain, coughs, colds, and other health conditions. Recent trends in the global market have witnessed a surge in the production of OTC sex wellness supplements and skin whitening products. All mass spectrometry experiments were carried out on an MT Explorer 30 (MTE30, MassTech Inc., Columbia, MD, USA) coupled to the DSAP source equipped with APCI and sESI/ESI modules. The data were acquired in MS and MS/MS (MSn) modes using the MODAS software. The qualitative data analysis was performed using ChromExplorer 1.5.1 software. For MS analysis total scan duration was less than 1 second. Result The results were validated with an Ultimate 3000 HPLC with an Orbitrap Fusion mass spectrometer (Thermo Scientific, Bremen, Germany). Samples of OTC creams, food supplements, and powders were procured from local drugstores and subjected to direct analysis using the MTE30 and HPLC-MS. Sample extraction and purification methods varied depending on the sample type. Our findings revealed the presence of illicit compounds in sex wellness products, such as tadalafil and lidocaine, as well as in intimate whitening creams, which are used for depigmentation purposes. Illicit substances including corticosteroids, hydroquinone, and its derivatives were identified and validated using laboratory-grade HPLC-HRMS instrumentation. The increasing demand for precise identification of illicit OTC products is driven by a global market expected to grow at an annual rate exceeding 12%. As market competition intensifies, it is anticipated that the use of illicit substances will rise in pursuit of greater market shares.Sensitive and specific instruments like mass spectrometry (MS) have demonstrated their effectiveness in untargeted analysis, enabling rapid and accurate screening, especially when compared to other analytical techniques such as spectroscopy or ion mobility systems.

* Ex æquo *best poster prize winner.*

MICROFLUIDIC DRIED BLOOD SPOT TECHNOLOGY FOR THE ANALYSIS OF COCAINE AND METABOLITES IN BLOOD BY UHPLC-MS

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The widespread consumption of cocaine (COC) has increased the need for effective analytical methods for its quantitation in biological matrices together with relevant metabolites such as benzoylecgonine (BEG) and cocaethylene (CET). To overcome the disadvantages associated with classic whole blood and plasma analysis, this study exploits the advantages of microsampling coupled to mass spectrometry for the design of an original bioanalytical approach for the simultaneous determination of COC and its main metabolites. In fact, microsampling strategies reduce sample volumes, simplify sample transportation and storage, minimize the invasiveness of sampling, while improving sample stability compared to traditional sampling [1]. In detail, whole blood microsample collection is carried out by an innovative microfluidic technology generating quantitative dried blood spots (qDBS), volumetrically accurate and independent of hematocrit. qDBS microsampling was coupled to ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS) and validated on blank fortified samples obtaining satisfactory results in terms of sensitivity (LLOQ: 2.3-4.0 ng/mL) and extraction yields (>85%).

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* Ex æquo *best poster prize winner.*

QUANTIFICATION OF SEVOFLURANE IN POST-MORTEM BLOOD SAMPLES BY HS-GC-MS

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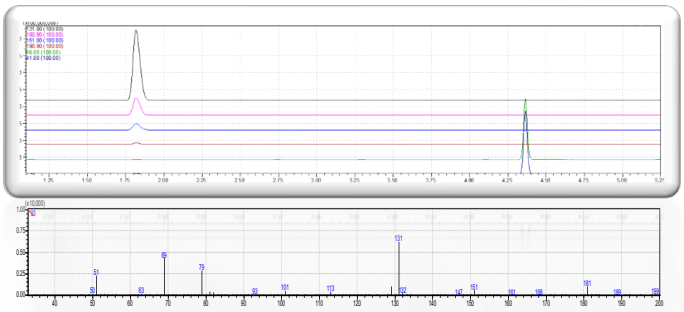

Fig. 1: Chromatogram and mass spectrum of SVF and internal standard in GC-MS in blood.

A simple and reliable headspace gas chromatography coupled to mass spectrometry (HS-GC-MS) method has been developed and validated for the determination of sevoflurane (SVF) in blood. This volatile compound may have forensic interest because it is dangerous and potentially lethal, causing cardiorespiratory depression, hypotension, and malignant hyperthermia [1]. Samples were prepared by dilution with an aqueous solution of internal standard followed by HS-GC-MS analyses. SVF and internal standard eluted in 6 min and were well resolved with no endogenous interference. Good linearity was observed in the range of 1.0 (LLOQ) to 304.0 µg/mL, with R= 0.999. Both the intra and inter-assay imprecision and inaccuracy of the method were ≤15%, according to the bioanalytical method validation guidelines [2]. The method developed have been applied to 20 negative controls (living and post-mortem samples), and blood samples of legal-medicine interest, that were subjected to SVF anesthesia days previous collection. The method was also successfully used for the detection and quantification of SVF in alternative matrices: brain, lungs in cases of unavailable blood sample. The present method is suitable for the identification and quantification of SVF in post-mortem blood, finding its application also in occupational medicine for monitoring anesthesia workers cannot be excluded.

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MICROEXTRACTION BY PACKED SORBENT (MEPS) COUPLED TO LC-MS/MS FOR THE ANALYSIS OF 41 ENDOGENOUS METABOLITES IN CELL CULTURE PELLETS AND CELL GROWTH MEDIA

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The characterization of metabolite profiles can be used as an important parameter in the study of metabolic pathways taking place in the cells including e.g., mitochondrial respiration, glycolysis, or lipid, amino acid and nucleotide metabolism, and their relationship with specific pathologies. Thus, measuring a wide array of metabolites can help elucidate physiological and pathological mechanisms, aid preclinical drug development and highlight potential therapeutic targets. An original liquid chromatography-tandem mass spectrometry (LC-MS/MS, triple quadrupole) method with electrospray ionisation (ESI) in both positive and negative modes was developed for the simultaneous quantitative analysis of 41 metabolites. Analytes were chosen among organic acids, amino acids, neurotransmitters and nucleotides commonly found in both cell pellets and cell growth media, and representative of those observed in the body's intra- and inter-cellular compartments. Sample preparation was miniaturised and semi-automated thanks to digitally programmed microextraction by packed sorbent (eVol®-MEPS) on a mixed-mode M1 sorbent (80% C8 and 20% SCX strong cationic exchange). MEPS follows a procedure similar to solid-phase extraction (SPE) but using a syringe-like device and a few milligrams of sorbent included into the modified syringe needle. This allows satisfactory and reproducible clean-up and preconcentration of both cell culture matrices, with minimal sample handling and greatly reduced sample, solvent, and energy consumption. The MEPS-LC-MS/MS method showed satisfactory linearity ($r^2 \leq 0.9984$), sensitivity (LOQ $\leq$ 5 ng/mL), and precision (RSD $\leq$ 9.8%). The method was successfully applied to 41 metabolites in cell pellets and cell growth medium from a line of immortalized mouse oligodendrocyte precursor cells, thus allowing investigations on the biochemical pathways altered in central nervous system pathologies especially occurring in infants.

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