Objectives and characteristics

- Give space to young mass spectrometrists, both from university and corporate environments, to present, share and discuss their work in front of an audience of experts

- Increase and enhance scientific communication through poster communications.

- Create the opportunity to increase the habit of oral presentation, enhancing the moments of questions and answers

- Registration is free for IMaSS members as is the format of the poster and the language in which it is displayed (English preferred).

- Instead of the poster, an electronic presentation, a prototype, etc. can also be proposed.

TARGETED AND UNTARGETED METHODS TO DETECT MICROPOLLUTANTS IN WASTEWATER AND AQUACULTURE

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Figure 1: Fish Farms

Organic and inorganic micropollutants are released daily into the aquatic environment through various pathways, posing a global threat because of their impact on human health and environmental ecosystems. In recent years, the problem of organic pollutants has become increasingly alarming, involving many classes of compounds such as pharmaceuticals, drugs, pesticides, odorous substances and personal care products. As a result, in order to assess the presence of micropollutants in aqueous matrices, it is necessary to implement increasingly effective analytical methodologies, such as combining high-resolution mass spectrometry (HR-MS) coupled with liquid chromatography (LC) and gas chromatography (GC) techniques. This study, conducted as a part of the H2020-MSCA-RISE-2020 SusWater Project, aims to monitor the presence of known volatile and non-volatile micropollutants through a suspected screening analysis (SSA) and to reveal emerging pollutants through a non-target analysis (NTA) approach in two different actual matrices, wastewater and fish farm water. Consequently, the monitoring of water quality thus obtained allows to tailor the proper restoration strategies aimed to water reuse through e.g combined chemical and biological strategies (fungi).

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Emerging contaminants in Coral reefs. Optimization of a SPME-LC-MS/MS method for the in-vivo determination of pharmaceuticals and personal care compounds in soft corals.

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The population growth in coastal areas and the inefficiencies in wastewater management pose the threat of emerging contaminants entering the marine environment. In the current literature, few studies examined the occurrence of pharmaceuticals and personal care compounds in tropical and coral reefs and their potential impacts on the associated fauna. The mayor problem in conducting research in these areas are related to the lack of research infrastructure and of suitable analytical method to be applied *in loco* without causing damage to this fragile and unique ecosystem. Starting from this basis we optimized a solid-phase-microextraction (SPME) and tandem mass spectrometry (LC-MS/MS) method to perform analysis *in vivo* in soft corals of the antibiotics Ofloxacin Sulfamethoxazole and Clarithromycin, the anti-inflammatory Diclofenac Propyphenazone Ketoprofen and Amisulpride, the neuroactive compounds Gabapentin-lactam, the beta-blocker Metoprolol and the antiepileptic Carbamazepine. Reproducibility was between 2.1% and 9.9% and method detection limits (MDLs) were between 0.2 and 1.6 ng/g. The procedure was then applied to establish a baseline of the occurrence of these compounds in the Maldivian archipelago. Colonies of *Sarcophyton s.p.* and *Sinularia s.p.* were sampled along an inner-outer reef transect in the Nilandhe atoll. 40% of the examined samples was found to be contaminated and the average contamination was 20.5±11.8 ng/g

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DEVELOPMENT OF A QUALITATIVE SCREENING METHOD FOR SMALL PEPTIDES IN DRIED BLOOD SPOTS (DBS)

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Dried blood spots (DBS) have been proven a valuable test matrix in doping control, enabling the analysis of several prohibited substances and their metabolites. Sampling does not require a trained phlebotomist, is less invasive, fast, and robust against manipulation. Moreover, samples can be shipped at room temperature and the support has the potential to stabilise easily degrading compounds in the sample. However, from an analytical perspective, the low sample volume (usually between 10 and 20 µL) represents a challenge in terms of method sensitivity and number of processes to apply.

Detection methods exist for the analysis of prohibited substances in DBS for anti-doping purposes. However, only a few methods[1],[2] target the group of so called “small peptides”. According to the World Antidoping Agency (WADA), small peptides are a class of substances that include peptides with a molecular weight lower than 3 kDa, such as the Gonadotropin Releasing Hormone (GnRH) and analogues or Growth Hormone Releasing Peptides (GHRPs) and GH fragments. Other compounds with a similar structure, though with a different pharmacological effect and mechanism of action, such as desmopressin, are also included into this class.

We present herein a qualitative initial testing procedure for the analysis of 27 small peptides and their metabolites in DBS. The analytes are extracted from the DBS (both cellulose and polymeric support) using first an extraction solvent containing water/acetonitrile/methanol and formic acid, followed by a second extraction with acetate buffer. To remove interferences, the combined extracts are further purified using solid phase extraction (mixed-mode, weak cation exchange). Analysis is performed using ultra-high performance liquid chromatography (UHPLC) combined with high-resolution mass spectrometry (Q-Exactive). The analysis time is 7.5 minutes, and the acquisition is performed in full scan positive electrospray ionisation, with the addition of some MS/MS experiment for a few compounds that are particularly challenging. Chromatograms are generated by extracting a 10 ppm m/z window.

The method proved satisfactory in terms of sensitivity (limit of detection between 0.5 and 20 ng/mL) and selectivity, with no interferences in negative control samples. Average extraction yield was estimated at 40% for cellulose and 20% for polymeric type DBS.

The use of UHPLC with high-resolution mass spectrometry was found to be an effective method for detecting small peptides in DBS at low levels. The use of the full scan detection approach facilitates future addition of analytes to the method. This is particularly relevant considering the constantly evolving cohort of substances that appear on the market and need to be detected.

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INTRATUMOR DISTRIBUTION OF DOCETAXEL DETERMINED BY MALDI- MSI IN LEIOMYOSARCOMA XENOGRAFT

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Leiomyosarcoma (LMS) is a common subtype of soft tissue sarcoma. Systemic therapies for advanced or metastatic LMS are disappointing. Eribulin (ERI) is an anti-microtubule agent clinically used in LMS therapy. Besides the direct effect on tumor cells it was shown to induce tumor vascular remodelling, potentially improving tumor drug delivery and treatment efficacy [1]. LM04 ERI-resistant LMS xenografts were treated with docetaxel (DTX, 30mg/kg i.v.), a second-line choice for LMS therapy, with or without ERI pre-treatment (1mg/kg, q7dx2). A Mass Spectrometry Imaging protocol was set up to visualize DTX spatial distribution in tumor slices. Explanted tumors were frozen in liquid nitrogen, cut, and mounted on a MALDI plate. The plate was sprayed with 1,5-diaminonaphthalene matrix containing paclitaxel (3µg/ml) as internal standard. A Q-Exactive HF hybrid quadrupole-Orbitrap MS coupled to an atmospheric pressure MALDI source was used. DTX distribution was assessed in 3 tumors/group. DTX tumor distribution resulted more intense and homogeneous in mice pre-treated with ERI (pixel-positive to drug 83.1% vs 65.5%, $p<0.01$, in tumors treated with the combination and DTX alone, respectively). Thanks to the developed imaging method, we demonstrate the ability of ERI to improve the tumor distribution of subsequently administered drugs. Efficacy studies are ongoing to demonstrate the "adjuvant" role of ERI in LMS treatment.

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INNOVATIVE APPROACHES FOR MICROPLASTICS DETECTION AND CHARACTERIZATION IN ENVIRONMENTAL MATRICES

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Microplastics (MP) represent a growing global environmental concern, with adverse effects on biodiversity and human health. As these particles, smaller than 5 um, are ubiquitous distributed in various terrestrial and aquatic ecosystems, their detection in different matrices is crucial for understanding the extent and effects of pollution [1]. MPs accumulate in various environments, including water, soils, oceans, beaches, and air, causing harm to marine and terrestrial fauna [2]. The inadvertent ingestion can cause harm to animals and humans, leading to a negative cascade impact throughout the food chain [3]. Moreover, MPs can also adsorb hazardous chemicals like pesticides and heavy metals, concentrating on them and releasing them into the organisms of those who ingest them, increasing the risk of adverse health effects [4].

This study proposes an innovative approach for the analysis of MPs in environmental matrices by combining infrared spectroscopy (Micro-FTIR and ATR-FTIR) and thermal desorption with SPME - gas chromatography-mass spectrometry (TED-GC/MS) [5], used to investigate the MPs levels in Lake Iseo (Italy).

We found MP residues in all analysed samples; thanks to the use of optical microscopy, we identified different types of MPs, including fibers, films, fragments, flakes, and pellets. The GC-MS method, was set for the analysis of three polymers: polyethylene terephthalate (PET), polystyrene (PS), and polyethylene (PE). The obtained results confirmed the presence of MPs in analysed samples and allowed us to estimate the total amount of these polymers in mass. Moreover, the IR analysis detected the presence of Nylon, PVC, Cellophane and the aforementioned polymers, highlighting a very heterogeneous distribution. In conclusion, this method, which used different analytical techniques, represents a new strategy for the identification of MPs in environmental matrices. In the future, we will improve the GC-MS technique for the identification of other polymers, such as Polyamide (PA), Polyvinyl chloride (PVC) and Polypropylene (PP).

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A rapid and sensitive UHPLC-MSMS method to simultaneously quantified five antibiotics in plasma of critically ill patients

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A major challenge in the management of critically ill patients in intensive care units is the microbial infections accounting for substantial morbidity, mortality, and costs. The extreme clinical conditions in these patients may alter the pharmacokinetic properties of the administered antibiotics, leading to inappropriate therapy. Thus, monitoring the circulating levels of antibiotics is crucial to define the effective schedule of treatment.

For this aim, we developed an UHPLC-MS/MS method for the simultaneous quantification of the antibiotics Tazobactam, Vancomycin, Meropenem, Linezolid and Piperacillin in human plasma. A low volume of plasma (50 µL) was purified by protein precipitation with acetonitrile and the supernatant was diluted and directly injected in a Vanquis Flex HPLC system (Thermo Scientific). Chromatographic separation was achieved by reversed phase within 5 minutes and the analytes were detected in ESI positive ionization in MRM mode using a TSQ Quantis Plus mass spectrometer (Thermo). Stable isotopes of each analytes were used as internal standards. The linearity ranges were 0.5 – 100 µg/mL for Tazobactam, Vancomycin and Meropenem, 0.06 – 90 µg/mL for Linezolid and 0.2 – 300 µg/mL for Piperacillin, suitable to measure the antibiotics plasmatic levels expected in clinical practise. The extraction recoveries for all analytes were above 70% with minimal matrix effect and all analytes were stable under all tested conditions. The within/between-run accuracy/precision satisfied the criteria set in the ICH M10 guideline for bioanalytical method validation. The methods have been successfully applied to investigate plasma concentrations of the five antibiotics in 240 critically ill patients enrolled in the clinical trial Abiokin. Further analysis will be carried out in all the other patients of the study, with the final goal to identify a strategy to optimize and monitor antibiotic therapy in infected critically ill patients.

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EFFECT OF POSTHARVEST 1-MCP TREATMENT ON THE VOLATILOME OF A NEW KIWIFRUIT (*A. chinensis)* GREEN-FLESHED VARIETY

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The landscape of Kiwifruit varieties has reached a turning point. Production of *Actinidia chinensis*, which are characterized by greater sweetness and aromaticity, is growing at the expense of that of A. deliciosa (*Zhong et al.*, 2021). The variety of Kiwifruit studied in this work (*Actinidia chinensis var. chinensis*, cv. AC45911) is the result of a breeding program designed to combine the sweetness and aromatic traits of yellow-fleshed *A. chinensis* varieties with the acidic component and the distinctive green colour of kiwifruit *A. deliciosa*. Dealing with a new variety, the main goal of this research was to investigate how the aromatic profile changes during shelf-life and after cold storage at 0.5 °C. A second important goal was to understand the influence of ethylene physiology (the ripening hormone in climacteric fruit, such as kiwifruit) on the aroma profile of AC45911 fruit. To achieve this second goal, treatment with ethylene inhibitor 1-methylcyclopropene (1-MCP) has been performed at harvest. Treatments with 1-MCP maintained significantly higher firmness values after one and three months of cold storage. In addition, 1-MCP affected soluble solids content and ethylene production, thus delaying fruit ripening. Volatile organic compound (VOC) profile obtained by GC-MS analysis enabled the quantification of 34 compounds belonging to the classes of alcohols, ketones, aldehydes, esters, and terpenes. The compounds with the highest relative abundance belong to the class of aldehydes (hexanal, (E)-2-hexenal and sorbaldehyde) and alcohols (hexanol and (E)-2-hexen-ol), which contribute to fresh, herbaceous and green notes (Atkinson et al., 2011). Interestingly, several terpenoids have been detected and may play an important role in conferring to AC45911 a distinctive aroma. Moreover, results preliminarily indicate a strong effect of 1-MCP treatment on several aroma components, the decreasing of which appeared to be delayed by the treatment.

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PHOTOCATALYTIC APPLICATION OF CARBON DOTS IN THE KNOEVENAGEL CONDENSATION: A MASS SPECTROMETRIC STUDY

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The Knoevenagel condensation is a variation of the aldol condensation between an active methylene compound and a carbonyl compound (aldehyde or ketone). This reaction has been widely used in the synthesis of various chemical compounds, including natural products, polymers, cosmetics, and drugs. The Knoevenagel condensation was typically catalysed by bases although the use of alternative "green" catalysts has been explored [1]. For this purpose, carbon dots (CDs) obtained from biowastes represents good candidates as catalysts [2]. The present study examined the photocatalytic application of CDs in the Knoevenagel condensation. The *p*-anisaldehyde was reacted with different methylene-activated compounds and the reaction was dual-mode monitored by electrospray ionization mass spectrometry (ESI-MS) in the presence of an UV irradiation source (365 nm, 9 Watt) to capture the ionic reactants, intermediates, and products of the reaction. Different reaction intermediates were intercepted based on the methylene-activated compounds used. Two different mechanisms were proposed: a classic base-catalyzed pathway passing through the formation of an aldol intermediate and an UV-promoted radical route resulting from a hydrogen atom abstraction from the C2-H moiety of the methylene group [3].

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THE IMPORTANCE OF CHIRAL COLUMNS: FURTHER INVESTIGATION FOR GC-MS ANALYSIS

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Identification of enantiomers is an important task in flavour, fragrance, and pharmaceutical fields, due to the possible different organoleptic and biological properties of the enantiomers of a chiral molecule [1]. Mass spectrometry (MS) is a powerful tool to identify compounds within a mixture and it is usually used coupled with separation techniques such as gas chromatography (GC) for the analysis of the volatile compounds. The combination of the information obtained with the mass spectra, with the linear retention indices calculated by injecting a homologous series of n-alkanes (C7-C30), and comparing them with the values reported in the literature, is used to identify volatile compounds in the absence of a reference standard. With conventional GC stationary phases, it is not possible to separate enantiomers, and MS spectra do not give information to discriminate optical isomers. For this reason, it is necessary to use enantioselective stationary phases to separate chiral compounds, and to use the linear retention indices in combination with MS spectra to identify the correct enantiomer. Cyclodextrin derivatives are the most popular chiral stationary phases used in GC to separate enantiomers in the flavour and fragrance field [2].

Important applications of chiral GC analysis is the authentication of the origin of raw materials and the detection of possible frauds or adulterations of natural products such as essential oils or other extracts.

In this study three different cyclodextrin derivatives were used as stationary phases for the evaluation of enantiomeric composition in a set of essential oils (EOs) analysed by GC-MS, including those of lavender (*Lavandula angustifolia* Mill.) and coriander (*Coriadrum sativum* L.), both containing linalool in very different enantiomeric ratios.

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ENDOBIOTICS QUANTIFICATION AND STRATEGIES FOR BIOANALYTICAL METHOD VALIDATION: THE CASE OF THE HIPPURIC ACID

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Accurate quantification of endogenous analytes plays a critical role in both clinical and pre-clinical studies. The European Medicinal Agency (EMA) and the Food and Drug Administration (FDA) guidelines [1], define the procedure for validating bioanalytical methods for the quantification of xenobiotic by mass spectrometry (MS) or other hyphenated techniques. The guideline assures the robustness and reproducibility of the proposed analytical strategy. Having analyte-free biological matrix when dealing with endogenous compounds detection/measurement is challenging, thus the validation of a quantitative method become a complex analytical issue. Hippuric acid, an endogenous molecule with a potential as biomarker of frailty in the elderly [2], takes center stage in this work. We evaluated different strategies to enhance the accuracy, precision, and reliability of MS quantitative analyses for endogenous compounds.

To mitigate the absence of blank matrices, four distinct strategies have been suggested in literature such as background subtraction, standard addition, the matrix surrogate, and the analyte surrogate [3]. In this study, we examinate two suitable approaches: the use of the matrix surrogate and the use of the analyte surrogate. Results indicate the successful implementation of our targeted MS methods, leading to a fully validated method for the quantitation of hippuric acid in plasma samples. The method demonstrated an excellent linearity (0.1–40 ng/ml in human plasma) in terms of accuracy (mean concentration of quality controls, N = 6, within ±15%) and precision (CV < 15%), while sample preparation was validated for recovery, matrix effect (CV < 15%), and stability (within ±15%) using the limits proposed by EMA guidelines. This study demonstrated the feasibility of establishing a comprehensive method validation for the analysis of endogenous compounds, exemplified by the case of hippuric acid, within the field of clinical studies.

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NEXT-GENERATION DRIED BLOOD SPOT (DBS) MICROSAMPLING COUPLED TO LC-MS/MS IN ANTI-DOPING TESTING

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LC-MS/MS is widely recognized as the golden standard for anti-doping testing of most substances prohibited by the World Anti-Doping Agency (WADA). Despite its unparalleled sensitivity and selectivity, the reliability of final results is still heavily dependent on the specific sampling and sample preparation steps. Microsampling, in particular, has recently attracted much interest, and dried blood spotting (DBS) has been recently approved by WADA in its official analytical workflows. In the DBS strategy, after finger pricking, a small amount of blood is deposited on a card (Fig. 1a) and dried. Sample drying increases analyte stability, avoids the need for special transport conditions and simplifies sample preparation. DBS sampling is minimally invasive and feasible in most situations without the need for specialized personnel [1]. Since classical DBS can be affected by some variability of sample volume and limited reproducibility, innovative strategies are under development to enhance their quantitative performance. One of the most interesting new volumetric DBS technologies is the Capitainer qDBS, which allows the accurate sampling of 10 µL of blood from a single drop (Fig. 1b) [2]. Afterwards, the analytes can easily be extracted using minute amounts of solvents and analysed by LC-MS/MS. In this study, the Capitainer qDBS approach has been used for the determination in blood microsamples of anabolic androgen steroids (AASs), one of the prohibited substance classes most frequently involved in illicit administration to athletes.

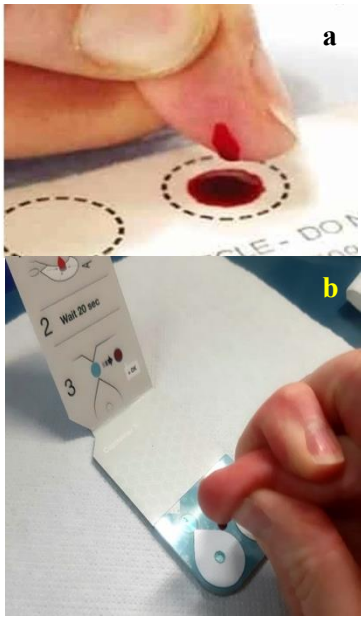

Fig. 1. (a) DBS and (b) qDBS.

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DIRECT CHARACTERIZATION OF ADULTERATED OREGANO LEAVES BY ATMOSPHERIC PRESSURE MALDI MASS SPECTROMETRY

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Economically motivated adulteration (EMA) of herbs and spices is a matter of great concern for consumers, producers, and regulators alike. EMA is a widespread practice that involves the deliberate addition of inexpensive substances to herbs and spices for economic benefit [1]. Accordingly, dried oregano appears to be the most vulnerable herb and it is suspected that approximately 48% of commercial samples may be adulterated [2]. Ambient mass spectrometry (AMS) methods analyze unprocessed or minimally modified solid samples enabling the rapid, economic and accurate analysis with minimal sample preparation [3]. In this project, a direct, rapid, and non-targeted method is proposed for classifying oregano samples using atmospheric pressure matrix-assisted laser desorption ionization mass spectrometry (AP-MALDI-MS) to distinguish between authentic oregano and its adulterants. This pilot study aimed to determine the chemical profiles of various oregano samples, including the most common adulterants such as sumac, olive, savory, strawberry tree, and myrtle. The AP-MALDI-MS fingerprints were acquired in both positive and negative ion modes and the two resulting data sets were pre-processed and submitted to supervised partial least squared discriminant analysis (PLS-DA) in order to discriminate the authentic oregano from the adulterants.

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Polyphenols profile in local chestnut variety by LC-MS/MS mass spectrometry

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Over the centuries, humans have carried out an intense artificial selection on the chestnut tree (*Castanea sativa Muller*), creating diversified genotypes capable of adapting and responding to the environmental, social, and economic situations of the moment. Currently, through the project 'Recovery of chestnut forest habitats in the chestnut groves of Camaldoli and Montalto', the attention has shifted to the study and valorization of nine local varieties of chestnut, specifically: 'Marrone del Casentino', 'Marrone di Stia', 'Marrone di Loro Ciufferna', 'Castagna di Mondistollo', 'Perella', 'Pistolese', 'Raggiolana', 'Tigolese' and 'Vitarina'. These nine varieties under study are fully integrated into the local cookbook, featuring in numerous traditional dishes with simple and tasty flavors, often born in times of severe food scarcity. In order to provide a nutritional characterization, 25 polyphenols were analyzed for each variety using 0.5 g of fresh weight of chestnut extracted with 25 mL of HPLC-grade 80% methanol (Sigma Aldrich, Milan, Italy). The 25 polyphenols were successfully identified and quantified by LC-MS/MS mass spectrometry (Sciex 5500 QTrap+), using an Information Dependent Acquisition (IDA) method with a selected reaction monitoring (SRM) transition per component used as survey scan and Enhanced Product Ions scan (EPI) for a mediated MS-MS spectrum identification. A Phenomenex Kinetex® Biphenyl 100 x 2.1 mm, 2.6 µm particle size column (Phenomenex, Torrance, CA, USA) was employed for the chromatographic separation. An elution gradient was performed using acetonitrile containing 0.1% v/v formic acid and MilliQ water with 0.1% v/v formic acid. The fresh weight of the different varieties follows the order 'Vitarina' < 'Castagna di Mondistollo' < 'Marrone di Stia' < 'Perella' < 'Raggiolana' < 'Tigolese' < 'Marrone Loro Ciufferna' < 'Pistolese' < 'Marrone del Casentino'. Significant differences were observed in the total polyphenol concentrations of the profiles of the chestnut cultivars under study. The sum of the 25 polyphenols analyzed ranged between 51018 ± 15600.2 ng g^{-1} FW in 'Vitarina' to 9803 ± 3454.8 ng g^{-1} FW in 'Marrone del Casentino'.

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LLE-LC-MS/MS METHOD FOR POLAR METABOLITES MEASUREMENT IN CORD PLASMA AND DRIED BLOOD SPOT SAMPLES

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A challenging issue in working with newborn and cord plasma samples is the limited sample volume. Consequently, it is important to be able to generate the most data from a limited quantity of biological matrix. An efficient method to separately extract polar and lipophilic metabolites, for targeted and untargeted metabolomics and lipidomics LC-MS/MS analysis, is liquid-liquid extraction (LLE). We performed LLE using methyl-tert-butyl ether, chloroform, water and methanol [1], [2]. We focused on the measurement of polar metabolites in cord plasma samples, particularly bile acids, kynurenines, acylcarnitines, polyamines and amino acids. It was evaluated how much the sample preparation affects the repeatability and linearity of the analysis and whether it involves loss or degradation of part of the metabolites.

An advantageous alternative to collect newborn biological material is using dried blood spot (DBS): the sampling is minimally invasive and the DBS allow a long-term stable preservation and easy transfer to the lab. We applied the same preparation protocol to the DBS samples to evaluate the possible interchangeability in the use of one or the other sampling modality.

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MASS SPECTROMETRIC ANALYSIS OF LOW MASS ORGANIC COMPOUNDS IN DIFFERENT ORGANS OF OLIVE TREE

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Poor quality irrigation water, could contain inorganic and organic contaminats such as salts, herbicides, pesticides, heavy metals, and personal care products, creating risks for crop quality [1]. For this reason, it is important to understand the uptake, metabolism, and distribution of these molecules/ions in plants. In this study, a completely randomized experiment was conducted for 102 days on *Olea europaea* cv. Cipressino (n = 5) plants irrigated with a solution containg a mix of contaminants (0.5 mg L^{-1} Cd, 0.5 mg L^{-1} $NiCl_2$, 60 mM NaCl, 1 mg L^{-1} CFN ^{13}C, 5 µg L^{-1} GLY and, 10 µg L^{-1}GLU). The focus of this work is on organic pollutants detection, and they were extracted from roots, leaves, and fruits following the method reported by Pierattini et al. [2]. Organic and their metabolites were determined by LC-MS/MS spectrometry (Sciex 5500 QTrap+) using information-dependent acquisition (IDA) method with Selected Reaction Monitoring (SRM) transitions (Table 1) optimized for each molecule as survey scan and MS-MS Enhanced Product Ions (EPI) spectrum acquisition. For each organic compound a calibration curve in the range of 1 to 256 ng ml^{-1} was performed. Chromatographic separations for Caffeine-^{13}C, theobromine and theophylline were performed with an Agilent PhenylHexyl 2x100 mm 2.7 µm column (Agilent Technologies) while for the analysis of Ammonium glufosinate, Glyphosate, MPP and AMPA Venusil HILIC 100x2.1 3 µm column (Agela Technologies) was used. As results the ammonium glufosinate metabolite (MPP) was detected at roots level (2.65±0.402 ng g^{-1} DW) while for leaves, stems and, fruits MPP was under the detection limit.

Table 1. SRM transitions and corresponding compound parameters for organic contaminants.

Compound	RT (min)	Q1	Q3	DP(V)	CE (EV)	CXP (V)
Aminomethylphosphonic acid (AMPA)	0.77	109.9	62.9	-90.0	-25	-9
Theobromine (THB)	1.58	181.10	138.1	-112.0	-26	-6
Ammonium glufosinate (GLU)	1.65	180.4	62.9	-91.0	-60	-6
Theophylline (THP)	1.77	181.10	124.1	-119.0	-27	-6
Caffeine (trimethyl-^{13}C) ($CFN^{13}C$)	2.13	198.10	140.1	-119.0	-28	-15
3-(hydroxymethylphosphinyl) propionic acid (MPP)	2.33	151.1	62.9	-78.0	-46	-3
Glyphosate (GLY)	2.52	167.9	62.9	-59.0	-31	-8

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PRESENCE OF EMERGING CONTAMINANTS IN BIOGENIC MATRICES USED IN AGRICULTURE AS FERTILIZER AND IN SOIL AND LETTUCE

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Sewage sludge is a typical by-product of our society and its reuse in agriculture is potentially an ideal solution for its disposal. The application of sludge and other biogenic matrices on overexploited agricultural land can bring great benefits due to their content of organic carbon, nitrogen, phosphorus and micronutrients. However, great attention must be paid to their potential adverse effects due to the presence of several classes of emerging contaminants (ECs), such as pharmaceuticals, antibiotics, personal care products and perfluorinated compounds. This may result in the transfer of ECs to the crops with potential risks for human health. The aim of this study was to investigate the presence of about 40 ECs in different substrates (sewage sludge, compost, digestate, pig and cow manure) and to assess ECs uptake in lettuce (*Lactuca sativa*) grown in soil mixtures containing the different substrates, as well as in the soil in which the lettuce was grown. The analysis of the ECs was performed by liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). The results showed different profiles of contamination in the different substrates investigated, with prevalence of fluoroquinolones, antibiotics for human use, in sewage sludge and of veterinary antibiotics in manure. Some antibiotics and plasticizers were found in *Lactuca sativa* indicating the possible uptake from soils to crops. The discharge of ECs into agricultural lands through the application of biogenic matrices can create stressful condition for the terrestrial ecosystems threatening its functioning. Moreover, the presence of antibiotics may also promote the spread of antibiotic resistance, a serious threat for human health. This study improved information on ECs presence in sludge and manure and their uptake in lettuce, with the overall goal to convert a potentially risky practice such as the application of biogenic matrices in agriculture into a safe process of circular economy.

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SCREENING OF BIOACTIVE MOLECULES ACTIVATING THE IMMUNE SYSTEM THROUGH ADVANCED MASS SPECROMETRY ANALYSIS

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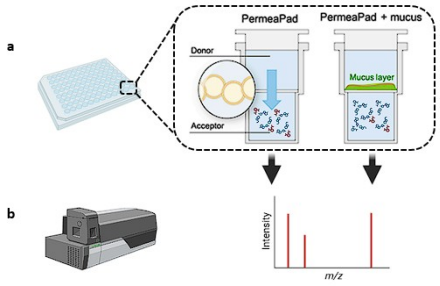

Fig. 1: a) Different permeation setups in the absence and presence of the cystic fibrosis mucus model. b) Detection of the target molecule through mass spectrometry analysis.

Quantitative analytical techniques are pivotal in both the pharmaceutical and biotechnological sectors. Among these, mass spectrometry (MS) holds the advantage to accurately detect complex and unstable molecules, even in confunding biological environments and it is hence used to quantify, for example, molecules within a biological environment as receptor-targeting compunds for specific therapeutic applications. In this work, MS was employed in tandem with high-performance liquid chromatography to optimize methods capable of identifying molecules acting on a specific cytoplasmic receptor, the AhR, that have a role in the pathological scenario such as cystic fibrosis (CF) and hence recently selected as possible terapeutic target [1]. In this study, the concentration of a set of nine molecules, targeting AhR, was quantified by MS (Shimadzu LCMS 8045). In particular, the MS method was optimized to be applied for molecules quantification in a commercial in vitro system (Permeapad®) for permeability estimation coupled with an artificial mucus model of the CF microenvironment [2]. Enabling the quantification of the molecules content, it was possible to compute the apparent permeability coefficients in in vivo-like CF-condition, thus investigating the impact of the pathological microenvironment on the capability of the selected molecules to reach their target (AhR). Importantly, the mass spectrometry results allowed to understand that CF microenvironment have a potent influence of AhR targeting, with 70% of permeability coefficient modified with reference to physiological-like in vitro conditions.

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Bacterial-Host communication:
A Permeability Study of Quorum Sensing Through a Mucus Model

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Biological systems inherently exhibit complexity characterized by a diverse array of components, interactions, and regulatory mechanisms. Understanding such intricacy necessitates advanced analytical approaches, among which mass spectrometry emerges as a crucial tool capable of significantly addressing these challenges. This technique enables both qualitative and quantitative analysis of numerous molecules, even at extremely low concentrations. Numerous molecules demand precise quantification, and in the case of unidentified compounds, their identification and characterization remain imperative; this is particularly true for Quorum Sensing (QS) molecules. Consequently, there is a growing need for the validation of mass spectrometry methods[1]. QS, a bacterial communication system, have a pivotal role in regulating antibiotic resistance in *P. aeruginosa*, while also acting in the interkingdom communication with the host, a phenomenon that remains poorly understood. In our study, we assessed the permeability of *P. aeruginosa* QS molecules, a previously unexplored investigation. To achieve this, we employed the Parallel Artificial Membrane Permeability Assay (PAMPA) coupled with mass spectrometry, facilitating quantification and permeability calculations. The five tested molecules were quantified using HPLC-MS/MS with a previously developed multiple-reaction-monitoring (MRM) method. All the molecules were found to be permeable, proving their capability to reach the AhR, a cytoplasmic receptor, as reported in *in vivo* data. To enhance the complexity of our model, we considered a frequently overlooked influential factor in molecule diffusion: mucus. Subsequently, we developed and evaluated a permeability model incorporating pathological mucus, modelling in particular the cystic fibrosis microenvironment. The mucus had an impact on the permeability of the molecules, especially on immune-triggers like Pyocyanin, providing an intriguing suggestion on how pathological mucus may mask this molecule, delaying the activation of host defence. Understanding the diffusion dynamics of *P. aeruginosa* QS molecules, as elucidated by mass spectrometry, holds promise for uncovering novel pathways for pharmacological intervention against this antibiotic-resistant pathogen.

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STUDY OF THE CANNABINOID AND TERPENE PROFILE AND DETERMINATION QUANTITATIVE OF Δ9-TETRAHYDROCANNABINOL (THC), CANNABIDIOL (CBD) AND CANNABINOL (CBN) IN MARIJUANA SAMPLES AND HASHISH SUBJECTED TO JUDICIAL SEIZURE.

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A Cannabis remains the most used narcotic substance worldwide. The illegal cultivation of Cannabis is a phenomenon with global impact that involves almost all countries, both European and non-European. The first objective of this study was to evaluate the presence of the components of main phytochemical interest of Cannabis sativa L. such as cannabidiol (CBD), Δ9-tetrahydrocannabinol (THC) and cannabinol (CBN), in samples of dry plant material and hashish subjected to judicial seizure.Quantitative analysis was performed with the Agilent 7820A GC gas chromatograph equipped with a FID detector (GC-FID). Most samples in this study reported low CBD/THC ratios indicating possible use for psychoactive effects and low CBN/THC ratios indicating freshness of the plant. CBD/THC ratios ranged from 143,982 to 0,010 in 2021, from 45,664 to 0,008 in 2022 and from 234,125 to 0,012 in 2023; CBN/THC ratios ranged from 320,395 to 0,007 2021, from 45,659 to 0,001 in 2022 and from 52,673 to 0,001 in 2023. Finally, the other ratio that was evaluated was (THC + CBN) /CBD which gives, as a result, useful values for distinguishing the phenotypes. Almost all of the samples demonstrated a phenotypic ratio >1, therefore the material can in all respects be classified as a narcotic hemp type. This confirms a widespread penetration and diffusion in the country of trafficking and possession of products that are marketed illegally for almost recreational use. In order to study the terpene profile of the samples, analyzed with GC-FID, a search was carried out for an extraction method that could allow for the most efficient qualitative identification of the cannabinoids and terpenes present. A sample of hashish and marijuana with a high THC content (on average above 25%) and which presented, following the GC-FID analysis, a chromatographic trace with peaks of CBD and CBN were chosen. These samples were subjected to three tests, with the three extraction solvents, different in polarity, which are most commonly used to extract cannabinoids and terpenes: ethanol for a polar extraction, a mixture of hexane and ethyl acetate (9:1, v/v) for a non-polar extraction and a mixture of hexane and ethanol (7:3, v/v) for a mixed polarity extraction. Polar solvents are the best for cannabinoid extraction, while the most suitable method for a more complete extract (cannabinoids and terpenes) of all active compounds is a mixture of polar and non-polar

solvents, such as n-hexane and ethanol. Qualitative analysis was performed with the Agilent GC gas chromatograph 7820A coupled to an MSD 5975 mass spectrometer, operating in electron ionization mode (70 eV) (GC/EI-MS). Derivatization of cannabinoids prior to analysis was necessary to avoid decarboxylation of acidic forms (THCA, CBDA, etc.) in the heated injection port and trimethylsilyl (TMS) derivatives proved suitable for this purpose. A total of 1 µL of each sample was injected in splitless mode into the GC/MS, and the acquisition mode was set to a full scan with a mass scan range of 40–550 m/z. The total analysis time was 20 min. The retention time of the analytes were: THCV-TMS, 14.624 min (selected ions: 358, 343, 315 m/z); CBD-2TMS, 15.106 min (selected ions: 390, 337, 301 m/z); CBC-TMS, 15.782 min (selected ions: 303, 246, 371 m/z); THC-TMS, 16.260 min (selected ions: 371, 386, 315 m/z); CBG-2TMS, 16.652 min (selected ions: 337, 391, 460 m/z); CBN-TMS, 17.120 min (selected ions: 367, 382, 310 m/z); THCA-A-TMS, 18.409 min (selected ions: 487, 488, 502 m/z); terpenes, on the other hand, come out roughly in the m/z range from 4 to 11. The identification of the cannabinoids and terpenes present was performed by comparison with the mass spectra reported in the NIST14 Mass Spectral Library.

UNVEILING THE DOMINANT ENANTIOMER OF 3MCPD ESTERS USING INDIRECT MASS SPECTROMETRY APPROACHES BASED ON GC-MS

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3-monochloro-1,2-propanediol (3MCPD) and its fatty acids esters are foodborne and thermally induced contaminants formed during the manufacturing process at high temperatures of vegetable oils and different foodstuffs [1]. In 2013, the International Agency for Research on Cancer classified 3MCPD as possibly carcinogenic [2], and the European Food Safety Authority published a scientific opinion on human health risks due to the presence of 3MCPD fatty acid esters in food [3], updating the tolerable daily intake to 2 µg/kg bw per day in 2018 [4]. Interestingly, 3MCPD exists as two enantiomers that appear to exhibit different biological activities: R-3MCPD has demonstrated kidney toxicity, while S-3MCPD has shown an antifertility effect in males [5]. Currently, the health risk assessment is conducted using the concentration levels expressed as the sum of the two enantiomers, and little information is present in the literature about the enantiomeric composition of 3MCPD in food products.

We propose a combination of two indirect GC-MS methods designed to evaluate both the total content of 3MCPD esters after hydrolysis and the ratio between the two enantiomers. We analyzed 23 vegetable oils, 1 fish oil, and 3 margarines. We quantified 3MCPD esters within the limits established by the European Union in the range of 0.044 to 1.435 µg/g. The two enantiomers, when detectable, were present in a 1:1 ratio.

Given these premises, if the two enantiomers can be separated and quantified, the health risk assessment can be revised and done specifically for the R-3MCPD and S-3MCPD. More studies on the toxicity of the two enantiomers are needed to determine the toxicity of each single enantiomer. In the future, we will apply these approaches to food samples as well to verify whether the ratio remains constant or changes under specific conditions.

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TARGETED LC-MS/MS MEASURMENT OF NOVEL BLOOD MARKERS OF EAAS DOPING IN VOLUMETRIC ABSORPTIVE MICROSAMPLING (VAMS)

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Volumetric Absorptive Microsampling (VAMS) is a recent technique used to obtain dried specimens of biological fluids that could represent a valid alternative to urine, whole blood and serum sampling for clinical measurements in the future [1]. The aim of the present work was to develop a UHPLC-MS/MS method for measuring the circulating levels of clinically relevant endogenous steroid hormones and phase II metabolites from dried blood microsamples collected with VAMS technology. Chromatographic separation was obtained by optimizing a 14 min multi-step gradient and employing fully-porous C18 analytical column. Ammonium fluoride was added to both aqueous and organic mobile phases to reach the high sensitivity level needed for measuring circulating levels of target steroids in 30uL dried blood microsamples. Different solvents were tested for improving the extraction of selected analytes from VAMS polymer. The method was validated in accordance with ISO 17025 requirements for quantitative methods and finally applied to real samples in order to evaluate the stability of steroidal compounds stored at different conditions for up to 100 days. The optimized chromatographic conditions allowed to efficiently separate all isomeric isobaric steroids included in the monitored panel and guaranteed a sufficient sensitivity for detecting endogenous hormones at low pg/mL level. The performed validation protocol, including the assessment of selectivity, matrix effects, extraction recoveries, quantitative performance (trueness, repeatability, intermediate precision, combined uncertainty, linearity range, LOQ), carry-over and robustness gave satisfactory results. The analysis of real samples highlighted the absence of significant differences in measured steroid concentrations when VAMS samples were stored at room temperature, 4°C, -20°C and -80°C for up to 100 days and subjected to up to 3 freeze-thaw cycles. The developed method proved to be suitable for steroid measurement in dried blood microsamples collected on VAMS support (30uL format). This innovative approach could represent a valid alternative to classic bioanalysis in clinical laboratories, especially for quantitative purposes in the pediatric field as a more reliable solution than DBS.

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World Anti-Doping Agency (WADA), project number 20D02FP, “Use of Volumetric Absorptive Microsampling (VAMS) for the quantification of novel blood markers of EAAS doping”

DEVELOPMENT OF LC-MS AND GC-MS METHODS FOR THE IDENTIFICATION OF GLUCOSINOLATES AND ISOTHIOCYANATES

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Glucosinolates are secondary metabolites of plants, which are found in very high concentrations particularly in the Brassicaceae (or Cruciferae) family and are known to have beneficial effects on the human body[1]. This is why interest in these compounds for the development of nutraceuticals and food supplements has become increasingly important in recent years. Within the plant matrix is also present the enzyme myrosinase, which under mechanical stress or due to temperature is activated and breaks down the glucosinolates, favouring the formation of isothiocyanates. The aim of this work was to develop mass spectrometric methods to obtain the selective identification of both intact glucosinolates and their isothiocyanates, with the further aim that these methods would be applicable to a variety of species belonging to the same family. In the case of the glucosinolates, it was necessary to block the action of the enzyme myrosinase, which is why in the sample preparation the samples were treated with liquid nitrogen. A method was then constructed in LC-MS, firstly performing a precursor ion scan, and then an MRM, in order to identify the masses corresponding to glucosinolates, supported by a literature search[2]. The precursor ion scan experiment provided a selective analysis of the class of compounds, also allowing a semi-untargeted approach to search for unknown or less abundant glucosinolates. Subsequently, high-resolution analyses were performed using an Orbitrap Fusion Triibrid UHPLC to confirm the masses found. In the case of isothiocyanates, the purpose was precisely to promote the action of the enzyme, so liquid nitrogen was not used, and different conditions were tested. A dichloromethane extraction was also performed to be able to perform GC-MS analysis with direct infusion, as isothiocyanates are volatile compounds. The next steps could be to optimise the proposed methods and to validate them, improving the quantification part; furthermore, a study on the correlation between plant maturation and the concentration of the compounds of interest would be desirable.

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Digestion protocol development for β-lactoglobulin characterization with nSI nanoHPLC-HRMS

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Dental calculus has recently emerged as a matrix of particular interest for the study of the ancient human oral microbiome and associated pathologies, subject's diet etc. In addition to macro/microscopic analysis of this matrix, the study of the proteins preserved in it provides information on the types of food intake (e.g. milk, cereals) [1]. A project to identify food sources by proteomics of dental calculus showed that lactoglobulin (BLG) is one of only two milk proteins, along with alpha-S1-casein, found in this matrix [2]. This low abundant whey protein, BLG, is not produced by human or bacteria, is specific to milk and, can be traced to the type of milk consumed through its species-specific isoforms. Sample pre-treatment protocols for proteins analysis are often based on a bottom-up approach in where the aim is to break down the mineral matrix, to reduce and to alkylate proteins, to extract and to digest them for a subsequent ESI-HPLC-MS analysis. Starting from peptide fragmentation, activated by CID or HCD modalities, it is possible to identify unique peptides belonging to food proteins using software and databases such as Protein Prospector and uniprot. The choice of extraction protocol, instrumental and data analysis parameters is an extremely complex process on which the final data and their subsequent interpretation are strictly dependent. It is therefore essential to start by studying known standard proteins to investigate which pre-treatment parameters maximise peptide identification. Moreover, sample pre-treatment requires multiple and unavoidable steps in proteomics by generating a long pre-analytical step. It is common to start by using a standard protein in order to evaluate greater digestion parameters for real applications. Attention was be made in proteins concentration, time and temperature of reduction/alkylation reagents, protein/enzyme ratio and working pH. The aim of this work is to evaluate the pre-treatment steps by studying the amount of trypsin, dithiothreitol (DTT) working temperature, DTT/ iodoacetamide (IAA)/ trypsin reaction time. A nano HPLC coupled with nSI HRMS Orbitrap Fusion and a C18 column were used to separate BLG peptides. An early data dependent acquisition (DDA) method was developed in order to verify the most efficient sample pre-treatment method in terms of number of peptides available using Proteome Prospector software and a tandem MS method focused on most specific and intense BLG peptides, which will be used to identify and quantify BLG in dental calculus. An initial application to real samples was attempted.

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VOCs PROFILES OF DEHYDRATING GRAPE BERRIES ARTIFICIALLY INOCULATED WITH *BOTRYTIS CINEREA*

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The addition of partially dehydrated grapes to enrich must composition for producing dry/sweet wines with a complex aromatic profile represents a traditional practice in several regions of the world, and nowadays it is facing a stage of renewed interest (e.g., Amarone). However, the optimal environmental conditions for the development of *Botrytis cinerea* [1], the causal agent of grey mould and responsible for significant grape losses, can be easily recreated inside the grape dehydration chambers. In this context, and considering the need of applying sustainable strategies to prevent the spread of *B. cinerea* infection and reduce grape decay, sensors can be used to monitor its presence in dehydration chambers. Few published papers [2, 3] report specific quanti-qualitative alterations of fruit volatile organic compounds (VOCs) profile in response to *B. cinerea*, and this knowledge can be addressed to the development of specific VOC sensors, which can be used for an early detection. With this goal and in order to deepen the understanding on VOCs specifically induced in berries artificially inoculated with *B. cinerea*, grape samples of Sangiovese and Corvina cultivars were collected after 0 (at harvest) and 40 days of controlled dehydration. Homogeneous intact berries with pedicels were selected and analysed as such and following (i) artificially inoculation with a spore suspension of *B. cinerea* (10^5 spores ml^{-1}) or (ii) mock inoculated by using the same volume of growth medium (control). After 5 and 40 days of incubation at 16 °C, VOC profiles have been analysed by GC-MS. Preliminary results have shown that inoculated berries appear to emit significantly higher levels of a set of primary, secondary and aromatic alcohols, some of which are already reported as correlated with *B. cinerea* infection (e.g., pentanol, isopentanol, hexanol, 2-hexen-1-ol, and 3-hexen-1-ol), while others (butanol and isobutanol) are not mentioned as infection markers yet. Moreover, the dynamics of the emission of some of these compounds

during the *in vitro* development of *B. cinerea* has been also studied. Setting up sensors capable of detecting the identified volatile markers inside dehydration chambers represents an ambitious goal for improving grape dehydration process in terms of yield and sustainability (key factors for wineries) by allowing for the early detection of *B. cinerea* and reducing spoilage via targeted adjustments of the dehydration conditions.

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Investigation of climate change effects on *Lepidium sativum*: a mass spectrometry-based metabolomics approach

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In the last century, the global average temperature has increased by 1.1°C, resulting in changes in precipitation, soil composition and properties (1,2), leading to a reduction in crop yields and alterations of the ecosystems. Understanding how climate change affects metabolism in plants could provide us with further insight into plant physiology, plant uptake of both nutrients and pollutants from soil, and, consequently, human exposure to pollutants. Given these bases, the aim of the present study was to identify which metabolic pathways were affected by a specific stress condition related to climate change through the detection of altered levels of metabolite contents in plants. In this study, twenty seedlings of *Lepidium sativum* were grown for seven days in presence of stress conditions (low temperature (4°C), absence of light, drought, irrigation with acidified water, irrigation with high-salinity water), control were treated with MilliQ water. We characterised the plant metabolic alterations in leaves and stems (total of 40 samples) using high-resolution mass spectrometry (Orbitrap Q Exactive, Thermo Scientific) coupled with liquid chromatography separation (Agilent Technologies, 1200 Series). The obtained data was analysed with Compound Discoverer Software (Thermo Scientific) for compound annotation, while the pathway analysis was performed using Metaboanalyst. Among the 129 metabolites accurately identified, 93 of them were significantly altered in treatments in comparison to the control group. The most impactful conditions were the absence of light and the drought, leading to more than 50 metabolites being significantly altered in both leaf and stem. Our results showed how plants change their metabolism to respond to abiotic stress, resulting in the alteration of five different pathways (lysine degradation; arginine and proline metabolism; arginine biosynthesis; glycine, serine, and threonine metabolism; alanine, aspartate, and glutamate metabolism). The proposed strategy paves the way to a comprehensive understanding of the impacts of climate change on metabolism and its implications for ecosystems and human health, creating a powerful bridge between metabolomics and ecology.

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Building and testing an in-house database of food intake biomarkers to uncover the link between diet and frailty in elderly

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Food-intake biomarkers (FIBs) are molecule derived from food intake and digestion with the potential to define associations between dietary habits and the risk of developing disease, including frailty: Failty is a geriatric syndrome leading to higher vulnerability of older adults to environmental stressors [1]. Several studies pointed out that a diet rich in fruits and vegetables is correlated with healthy aging. Therefore, there is an urgent need of reliable biomarkers to identify populations at risk of frailty when this syndrome is still subclinical and intervention can be effective. The aim of this work is the construction of an *in-house* FIBs database to evaluate the association between measurable FIBs, dietary habits and frailty development. FIBs database was constructed from literature-search of molecules related to the intake of specific foods (e.g.phytochemicals, methylxanthines, fish- and meat-derive, artificial sweeteners, preservatives) and phase I-II metabolites. Monoisotopic masses of each FIBs were queried against four different databases: the Human Metabolome Database, FoodDB, Phenol Explorer and Exposome Explorer, obtaining more than 2000 possible FIBs. The database was cleaned removing duplicate masses and considering only those FIBs validated by MS/MS spectra. The final version of our *in-house* database included 976 FIBs.The database structure accommodate monoisotopic mass and the most common adducts (+Na, +K), the HMDB ID, the molecular classes, and food classes. Database performance was tested on plasma samples of 130 older adults (65 Fit and 65 Frail) without dietary intervention, by flow-injection analysis-mass spectrometry (FIA-MS). EASY-FIA [2] tool was used to pre-process and annotate data using the *in-house* FIBs database. We detected 83 FIBs that capture a picture of individual dietary habits, representative of an high prevalence of plant-derived foods intake (FIBs in > 50% of subjects) and low prevalence of fish, artificial sweaters and alcoholic beverages (FIBs in <50% of subjects). Overall, our database may act as tool to identify circulating compounds derived from diet potentially associated with the frailty onset, with value for counteracting frailty development.

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POLYPHENOLS PROFILE IN TOMATO (*SOLANUM LYCOPERSICUM* L.) CV. MICRO-TOM UNDER NICKEL STRESS

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Tomato (*Solanum lycopersicum* L.) is a largely commercialized crop. Its fruits are valuable for their nutritional properties as a source of antioxidant-beneficial molecules [1]. The essential element Nickel (Ni) can be delivered in excess in soil and crop plants by irrigation water contaminated by industrial activities [2]. In plants, Ni toxicity can increase the amount of ROS (reactive oxygen species) and cause oxidative damage. To fight ROS, plants can provide non-enzymatic antioxidant defense systems like polyphenols [3], [4]. To understand Ni's impact on tomato plants' physiology and nutritional properties, *Solanum lycopersicum* L. cv. Micro-Tom plants were cultivated in a hydroponic system (under controlled environmental conditions) containing half-strength Murashige and Skoog solution supplemented with 0 (control), 0.1, 0.2, and 0.4 mg L^{-1} of Ni. The solution was renovated after one week for 15 days. The polyphenols were extracted from approximately 0.3 g roots and leaves fresh material using 3 mL of 80:20 (v/v, methanol/MilliQ), shaken in the dark for one hour, centrifuged for 15 minutes at 4000 rpm, filtered through a 0.45 µm Whatman nylon filters and diluted 1:5 with MilliQ water before undergoing UHPLC-MS/MS analysis. A Sciex 5500 QTrap+ mass spectrometer (AB Sciex LLC, Framingham, MA, USA) equipped with a Turbo V ion spray source coupled to an ExionLC AC System custom-made by Shimadzu (Shimadzu Corporation, Kyoto, Japan) was used to study 9 known phenols. More in detail, 2 phenolic acids (vanillic acid and trans-ferulic acid), 1 caffeic acid derivates (chlorogenic acid), 4 flavonols and their derivates (kaempferol-3-O-glucoside, kaempferol-3-O-rutinoside, rutin), 1 flavanone (naringenin), and 1 flavone (apigenin) were analyzed. A Phenomenex Kinetex ® Biphenyl 100 × 2.1 mm, 2.6 µm particle size column (Phenomenex, Torrance, CA, USA) was used for chromatographic separation of polyphenols. Calibration curves ranged from 0.5 to 256 to ng mL^{-1}. The results showed that Ni was uptaken by the roots and translocated into the leaves, leading to a significant alteration in the flavone, flavonol, and flavonoid biosynthesis. In particular, the kaempferol-3-O-glucoside, kaempferol-3-O-rutinoside, naringenin, and rutin increased in treated plant organs. Data proved that Ni uptake has an impact on tomato phenolic profile.

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Investigating the ecological role of BVOCs emission from marine algae by advanced Mass Spectrometry tools

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Algae as plants emit Biogenic Volatiles Organic Compounds (BVOCs) and contribute significantly to the global budget of volatiles in the atmosphere, participating in aerosol formation. BVOCs are involved in algae growth, reproduction, and defence. They act as 'infochemicals' and may be effective in structuring biotic interactions and regulating key ecological processes (1,2). It has been hypothesized that anthropogenic micropollutants may interfere with the chemical communication processes that occurring between organisms even below the determined thresholds for toxicity (3,4). Evidence of chemosensory interactions have been collected both for pelagic and benthic aquatic systems but the information for algae is lacking (5,6,7). In this context, advanced mass spectrometry tools may provide a huge potential to further the understanding of the 'chemical language' of algae and the possible interference caused by anthropogenic chemicals. We therefore developed a method to underline the possible interference by employing SPME coupled to GC-MS in sim-scan acquisition mode. By preliminary tests, we successfully collected the fully volatilome profiles of 6 different marine algae while detecting the occurrence of 20 selected emerging contaminants. Optimization, validation and the preliminary data collected are discussed.

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ANALYSIS OF BENZOYLATION REACTION AS A DERIVATIZATION STRATEGY FOR QUANTIFYING METABOLITES IN HUMAN PLASMA

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One specific strategy to increase instrumental sensitivity of liquid-chromatography mass-spectrometry is the derivatization of the target analytes to increase their ionisation properties. Different reactions are possible according to the molecular structure, however in the last decades there were several reports on the use of benzoyl chloride as derivatization reagent when an amino or phenol group is present in the target molecule. The advantages of this strategy are many: very mild reaction conditions, short reaction time, and the possibility to synthesize the proper internal standard for each analyte by using the labelled version of benzoyl chloride. However, some limitations must be considered: the reagent cannot be removed, increasing matrix effect, and the internal standard cannot be added at the beginning of the process but only after derivatization. Thus, the following question arises: is the benzoylation reaction a good derivatization strategy for the analysis of metabolites extracted from human plasma? To address this matter, a group of selected analytes was chosen (some amino acids, some amino compounds, and molecules with a phenolic group). A wide range of physiological concentrations in plasma were also covered by these selected metabolites (from 0.01 µM to 100 µM). A pool of different benzoylated compounds was obtained, each with its own internal standard analogue isotopically labelled. After development of this method, the validation was achieved following the guidelines released by FDA[1]. Then, the new methodology was tested by analysing the reference material NIST SRM 1950 and comparing the concentrations found with those reported for the reference standard. Furthermore, for comparison the same plasma samples were measured with our methodology and with the Biocrates kit MxP Quant 500. The results showed a LLOQ of 0.003 µM for the lowest concentrated analytes, and accuracy and precision within the FDA guidelines. Moreover, the data indicate in many cases a good agreement between the concentrations obtained with our derivatization protocol and the reference standard with ratio values from 0.85 to 1.10.

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Non Target Analysis Approach for Uncovering Flavours in E-Liquids using Gas Chromatography-Mass Spectrometry

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In the last decade the use of e-cigarettes increase among young and adult people [1] and their popularity may be attributed to the availability of different flavours[2]. Most e-cigarette users, especially young people, prefer e-liquids with a fruit or sweet flavours over traditional tobacco flavours [3].The appealing flavours present in e-cigarettes contribute to their attractiveness and increase the willingness to try these products [4],[5].

Almost all flavouring compounds, used in e-cigarettes, are classified as Generally Recognized As Safe (GRAS) [6] and approved for oral consumption, but their safety for inhalation is not confirmed [7] as there are studies in the literature that suggest a correlation between respiratory toxicity and these compounds [8],[9]. To better understand the composition of e-liquids and assess the harmfulness related to their use, there is an unmet need for a validated non-targeted analysis (NTA) method that can identify the composition of e-liquids with certified accuracy. Here, we propose a NTA method for e-liquids screening using gas chromatography coupled to mass spectrometry detection (GC-MS). The method enabled the identification of the main volatile organic compounds with a signal-to-noise (S/N) ratio greater than 10, as well as the assessment of their concentrations through a direct comparison with the internal standard. The developed method was applied to the analysis of 23 e-liquid samples collected by the World Health Organization (WHO) from various countries. Among the samples, the most common flavours identified were butanoic acid ethyl ester and 3-hexen-1-ol, responsible for fruity flavour. Secondly, we identified ethyl maltol which gives a sweet flavour and ethyl diisopropylacetamide, a synthetic coolant, which provides an ice-cool sensation. The proposed strategy successfully characterized the flavours of 23 e-liquids sampled worldwide by the WHO. The main objective of this work is the development of a validated WHO's Standard Operating Procedures (SOP) for NTA analysis in e-liquids in order to use this method globally. This approach has proved to be a reliable testing method for ensuring the safety and

regulation of e-cigarettes and assessing the degree of agreement among independent laboratories in NTA of flavours

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Polyphenols profile in local chestnut variety by LC-MS/MS mass spectrometry

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Over the centuries, humans have carried out an intense artificial selection on the chestnut tree (*Castanea sativa Muller*), creating diversified genotypes capable of adapting and responding to the environmental, social, and economic situations of the moment. Currently, through the project 'Recovery of chestnut forest habitats in the chestnut groves of Camaldoli and Montalto', the attention has shifted to the study and valorization of nine local varieties of chestnut, specifically: 'Marrone del Casentino', 'Marrone di Stia', 'Marrone di Loro Ciufferna', 'Castagna di Mondistollo', 'Perella', 'Pistolese', 'Raggiolana', 'Tigolese' and 'Vitarina'. These nine varieties under study are fully integrated into the local cookbook, featuring in numerous traditional dishes with simple and tasty flavors, often born in times of severe food scarcity. In order to provide a nutritional characterization, 25 polyphenols were analyzed for each variety using 0.5 g of fresh weight of chestnut extracted with 25 mL of HPLC-grade 80% methanol (Sigma Aldrich, Milan, Italy). The 25 polyphenols were successfully identified and quantified by LC-MS/MS mass spectrometry (Sciex 5500 QTrap+), using an Information Dependent Acquisition (IDA) method with a selected reaction monitoring (SRM) transition per component used as survey scan and Enhanced Product Ions scan (EPI) for a mediated MS-MS spectrum identification. A Phenomenex Kinetex® Biphenyl 100 x 2.1 mm, 2.6 µm particle size column (Phenomenex, Torrance, CA, USA) was employed for the chromatographic separation. An elution gradient was performed using acetonitrile containing 0.1% v/v formic acid and MilliQ water with 0.1% v/v formic acid. The fresh weight of the different varieties follows the order 'Vitarina' < 'Castagna di Mondistollo' < 'Marrone di Stia' < 'Perella' < 'Raggiolana' < 'Tigolese' < 'Marrone Loro Ciufferna' < 'Pistolese' < 'Marrone del Casentino'. Significant differences were observed in the total polyphenol concentrations of the profiles of the chestnut cultivars under study. The sum of the 25 polyphenols analyzed ranged between 51018 ± 15600.2 ng g^{-1} FW in 'Vitarina' to 9803 ± 3454.8 ng g^{-1} FW in 'Marrone del Casentino'.

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Targeted-metabolomics of kynurenine pathway intermediates using LC-MS/MS: a reliable readout for assessing the potency of selective hIDO1 inhibitors in cells

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L-Tryptophan is an essential amino acid that undergoes metabolism through the kynurenine pathway (KP), with the key enzyme hIDO1 (indoleamine 2,3-dioxygenases 1). Targeting the KP shows promise in cancer management, leading to the development of several hIDO1 inhibitors. [1] Bioanalytical chemistry plays a crucial role in understanding the KP and exploring potential therapeutics. Profiling kynurenines is challenging due to their diverse properties. Quantitative liquid chromatography with tandem mass spectrometry (qLC-MS/MS) is a reliable and selective technique with high resolution and minimal interference. [2] Plasma, serum, and urine are commonly used biological fluids to measure hIDO1 activity by evaluating the kynurenine/tryptophan ratio. [3] Cell culture supernatants provide a physiological environment for in vitro cancer research, facilitating the assessment of drug properties. However, there is a limited number of published LC-MS methods for quantifying kynurenines in cell culture media, highlighting the need for further development. This abstract introduces a validated LC-MS/MS method for quantifying multiple kynurenines in cell culture media. The method was validated following the guidelines EMA, ICH, FDA, and then applied to study the effects of the selective hIDO1 inhibition on the kynurenine pathway in melanoma, breast cancer, and glioblastoma cell lines. It was also used to determine the in vitro efficacy of hIDO1 inhibitors. The proposed LC-MS/MS method is reliable, robust, and versatile, making it suitable for preclinical drug research and in vitro assays.

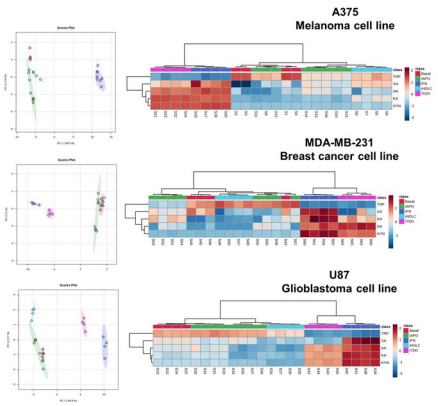

Fig. 1: Metabolic Insights: Selective IDO1 and TDO Inhibition.

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MS-IMAGING: IN SITU INVESTIGATION OF SECONDARY METABOLITES OF ORYZA SATIVA L. VARIETIES BEFORE AND AFTER COOKING

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Rice (*Oryza Sativa L.)* is an important cereal in human nutrition due to its richness in vitamins, minerals, and bioactive compounds. The processing steps to which paddy rice is subjected after harvesting, while on the one hand making it edible improving its sensory quality and cooking, on the other hand, from a nutritional point of view could result in the depletion of many micronutrients and phytonutrients that are concentrated in the germ and bran [1]. An effective processing technique to reduce cooking time of rice is parboiling, a hydro-thermal treatment that allows the water-soluble components of the germ and outer parts to diffuse within the caryopsis [2]. The aim of the work was the application of MALDI-MSI [3] to investigate the localization and migration of two different classes of rice micro-components after industrial processing and domestic cooking. The target analytes of this study were steryl ferulates and anthocyanins [4], which are known to provide positive physiological effects on human health [5]. The Oryza Sativa L. varieties studied were 'Carnaroli', a white rice, 'Oro', a white parboiled rice, 'Nerone', a black pigmented rice, and 'Venere', a black pigmented parboiled rice. About domestic cooking methods, all rice varieties were analyzed raw, after boiling in water and after steaming.

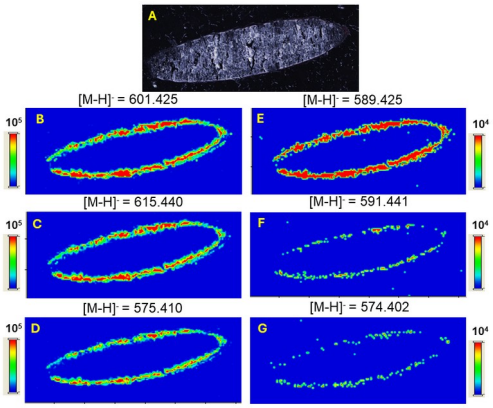

Fig. 1: Figure 1: Steryl ferulates ion images acquired on a Nerone rice seed section as uncooked. (A) Optical microscopic image, (B) Cycloartenyl ferulate, (C) 24-Methylenecycloartenyl ferulate, (D) campesteryl ferulate, (E) sitosteryl-ferulate, (F) sitostanyl ferulate, (G) caffeoyl phytosterol.

Spectra acquisitions with IMS were conducted on a MALDI-LTQ-Orbitrap XL and the ionic intensity of the signal associated with the different distribution of the analyte in the tissue was evaluated.

As regards steryl ferulates, the study allowed us to verify their localization in raw materials and observe a negligible migration towards the endosperm of the parboiled rice seed. Regarding anthocyanins, it was found that steaming or boiling pigmented rice provides migration of these compounds in the endosperm.

The results obtained provide a solid basis for the definition of future experimental designs for the study of rice secondary metabolites and for monitoring the effects of parboiling on their loss and redistribution during domestic cooking.

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TEZACAFTOR® IS A DIRECT INHIBITOR OF SPHINGOLIPID DELTA-4 DESATURASE ENZYME (DEGS)

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Background

We are currently investigating the off-target effect of Kaftrio, a triple drug combination (ETI: E = Elexacaftor, T = Tezacaftor and I = Ivacaftor) designed to be the main treatment for patients with cystic fibrosis, carrying F508del on at least one allele. We hypothesize that this drug might interact with a specific enzyme, delta-4 sphingolipid desaturase (DEGS), which converts dihydroceramides (dHCer) into ceramides (Cer). As previously demonstrated by our group [1], bronchial epithelial cells (BE), obtained from both CF and non-CF patients, show dhCer up-regulation when treated with ETI.

Method setup and analysis

For *in-vitro* DEGS activity assessment, we used a previously described protocol [2]. We analyzed Cer and dHCer using an LC-MS method, monitoring specific MRM transitions of the substrate and the product, after DEGS quantification by western blot analysis.

Results

We demonstrated that the accumulation of dHCer in BE increases with time, under prolonged ETI exposure, while the western blot analysis showed no reduction of DEGS expression. We proved that ETI inhibits DEGS activity, finding that Tezacaftor is the sole molecule responsible for the inhibitory effect, depending on the concentration and observing this effect at concentrations lower than those observed in CF patients undergoing ETI treatment. Further investigations on Tezacaftor safety should be envisaged, particularly for the use of ETI during pregnancy, breastfeeding and in the early stages of development.

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SBSE-HPLC-TANDEM MS BASED METHOD FOR MULTI-RESIDUE DETERMINATION OF PESTICIDES IN DRINKING WATER

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Pesticide residues water contamination represents a significant public and political issue due to their potentially harmful effects on environment, biodiversity, and human health even at extremely low concentrations. According to the European Council Directive 2020/2184 (ex 98/83/EC), limits for pesticide residues in water intended for human consumption are set at 0.1 μg/L for individual pesticides and 0.5 μg/L for the sum of all pesticides. Thus, the implementation of sensitive analytical methods for pesticides identification and quantification in aqueous matrix has become extremely important in the last decades. Considering the extreme variety of chemical and physical properties of pesticides, the greatest challenge is the development of multi-residue methods that allow monitoring of the greatest number of hazardous chemicals deemed as priorities by health authorities while respecting the minimum requirements of performance of the analytical method (2009/90/EC; DL 219/2010). The use of gas chromatography-mass spectrometry is a well-established tool for this purpose making possible to detect even parts per trillion for some pesticide compounds [1]. Moreover, LC coupled with mass spectrometry or tandem mass spectrometry has also received remarkable attention due to the spread over the years of polar, thermolabile, low volatile pesticides not fit for GC analysis [2]. Among the variety of techniques for the preconcentration and isolation of organic compounds from aqueous matrix, Stir Bar Sorptive Extraction (SBSE) proves to be a simple, automatable, and both GC and HPLC compatible technique [3].

Herein, we aimed to set up a multi-residue method for the determination via HPLC- tandem MS of about 200 pesticides in mineral water based on sequential SBSE. The method was tested in fortified milliQ water samples with a lower limit of quantification (LOQ) of 20 times lower than that required by European directive for most of the investigated pesticides. The proposed SBSE-HPLC tandem MS method was applied on real mineral water samples to investigate its applicability as routine tool for control analysis of pesticide residues in drinking water.

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EPS-URINE DERIVED EXTRACELLULAR VESICLES: METABOLOMIC PROFILING FOR PROSTATE CANCER DIAGNOSIS AND PATIENTS' STRATIFICATION

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Given the reduced specificity of currently used diagnostic tests in Prostate Cancer (PCa) [1], there is an urgent need for new biomarkers that can help to more specifically and sensitively diagnose this disease and stratify patients. Urinary expressed prostatic secretion (EPS) represents an interesting source of potential biomarkers for PCa [2]. The protein cargo of EPS-urine derived exosomes has been widely investigated [3], but their metabolomic fingerprint is not yet fully characterized. Considering the peculiar prostate metabolism [4], we aim at filling this gap by providing an untargeted LC-MS/MS metabolomic profiling of EPS-urine-derived extracellular vesicles (EVs) from PCa patients with different cancer grade. EVs were isolated through ultracentrifugation from 68 patients undergoing prostate biopsy. Patients were stratified in 4 groups: 17 patients with negative biopsy, the remaining 51 with low (n=17), intermediate (n=17) and high-risk (n=17) PCa according to EAU risk groups, respectively. We decided to apply a global metabolite extraction protocol to obtain both the polar and apolar metabolites. We analysed the extracted metabolites through liquid chromatography coupled to tandem mass spectrometry. Two different experimental approaches were combined: Data Independent Acquisition (DIA) and Hermes-implemented Data Dependent Acquisition (DDA). The innovative use of the two experimental workflows allows successfully characterizing EPS-urine derived EVs metabolome. Hermes-DDA design was used as complementary approach to confirm uncertain annotations from DIA analysis [5], thus increasing the number of final identified metabolites. We were able to identify 111 apolar metabolites. Among the six lipidic classes specific for our EVs, ceramides and phospholipids result to be reduced in patients with high-risk cancer. Lipid enrichment analysis highlights some recurrent terms associated to cancer "lipogenic" phenotype in high-risk patients. Regarding the polar metabolite fraction, we succeeded in identifying 125 metabolites. Aspartic acid and uric acid were highly abundant in patients with low-risk cancer and then decreased with the degree of malignancy ($p<0.05$). An in-depth pathway analysis revealed that in the context of high-risk patients compared to those with BPH and low-risk disease, certain metabolic pathways experienced significant alterations ($p<0.05$). Specifically, glycine, serine, and threonine metabolism, cysteine and methionine metabolism, and arginine biosynthesis emerged as the most notably affected metabolic pathways. Our results confirm the metabolic phenotype associated to PCa. More interestingly, our research provides fresh insights into the metabolic fingerprint of PCa. We have uncovered potential metabolic markers within EVs that hold significant importance in the development and progression of PCa. EVs are recognized as pivotal agents in cancer pathogenesis due to their capability to transport molecules to distant locations from the tumor's origin. Our findings indicate specific metabolites within EVs that may play a pivotal role in driving the aggressiveness and advancement of PCa. This newfound knowledge has the potential to pave the way for innovative diagnostic and prognostic tools, ultimately improving the clinical management of PCa patients.

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LC-MS/MS METHOD DEVELOPMENT FOR THE QUANTIFICATION OF KAFTRIO IN THE BREAST MILK OF CYSTIC FIBROSIS PATIENTS

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Kaftrio is a triple therapy recently approved by the FDA and EMA containing the active principles Ivacaftor, Tezacaftor, and Elexacaftor for the treatment of cystic fibrosis (CF) patients aged 6 years and older who have at least one *F508del* mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. As a result of the high efficacy of this treatment in improving life quality, CF women patients are more willing to seek pregnancy, but informed decisions about breastfeeding while taking the medication are currently difficult to take due to the lack of data about both the amount of drug potentially transferred into breastmilk and infants' health risks. For this reason, the aim of this study has been then to develop and optimise an LC-MS/MS method for the detection and quantification of Kaftrio and its metabolites M1-Ivacaftor, M6-Ivacaftor, M1-Tezacaftor and M23-Elexacaftor in breast milk (BM). Given the extreme complexity and variability of the matrix composition, both liquid-liquid extraction (LLE) and protein precipitation (PP) combined with a clean-up step using a hydrophilic polyvinylidene membrane filter plate for lipids removal have been investigated as sample preparation techniques, with the PP protocol resulting in having the best outcomes in terms of volume of breast milk required, chromatographic performance, recovery efficiency (RE%) and matrix effect (ME%). The chromatographic separation has been performed on a reversed-phase C4 column in gradient elution mode using acetonitrile-0.1% formic acid and water-0.1% formic acid as mobile phases, while the quantification has been carried out using deuterated internal standards and multiple reaction monitoring (MRM) via a triple quadrupole mass spectrometer. A method validation has been attempted following EMA guidelines [1] but further work is required to improve both precision and accuracy as well as to investigate possible interferences from co-administrated drugs (antibiotics).

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