Book of Abstracts

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Agenda

Thursday 25th May

12:00 – P***oster installation / welcome feast - Aula vittime innocenti di tutte le Mafie***

13:30 – ***Registration - Aula Magna Aldo Cossu***

14:00 – ***Greetings and opening ceremony***

Presidente della Divisione Spettrometria di Massa SCI - Prof.ssa Giuliana Bianco
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Thursday 25th May– session 1: Biomedics

Chairmens: Dott. Federico FANTI & Dott. Giovanni VENTURA

14:30 – **Plenary Lecture** – *Region-specific lipid markers of plaque outcome revealed by mass spectrometry imaging of human carotid atherosclerotic plaques* - Francesco GRECO - Fondazione Monasterio Pisa

15:00 – **OR 1** – *Mass spectrometric characterization of methyl carbamates of phosphatidylethanolamines and phosphatidylserines generated in the mitochondrial lipid extracts of mouse embryonic fibroblasts* - Andrea CASTELLANETA - Università degli Studi di Bari "Aldo Moro"

15:13 – **OR 2** – *Mass spectrometry in urinary cotinine determination as a passive tobacco smoke biomarker* - Chiara MACCARI – Università degli Studi di Parma

15:26 – **OR 3** – *LC/MS-MS assessment of CFTR Modulators Concentrations in Biological Fluids from Adult and Children with Cystic Fibrosis* - Matteo MUCCI - Università degli Studi di Chieti – Pescara "G. d'Annunzio"

15:39 – **OR 4** – *Capillary Electrophoresis-Mass Spectrometry for Comprehensive Profiling of Free and Conjugated Estrogens* - Luciano CALLIPO - AGILENT

15:52 – **OR 5** – *BAG3 in glucose homeostasis: proteomics characterization of BAG3 knockout in pancreatic β-cells – un update* - Federica DI MARCO - Università degli Studi di Chieti – Pescara "G. d'Annunzio"

16:05 – **16:20 Coffee break**

Giovedì 25 – session 2: Environment and Cultural Heritage

Chairmen: Dott.ssa Flavia PAGANO & Dott.ssa Sara PALMIERI

16:25 – **Keynote1** – *Mass spectrometry for the characterization of organic materials: from ancient to contemporary artworks* - Elena RIGANTE- Università degli Studi di Bari "Aldo Moro"

16:45 – **OR 6** – *Natural rubber: a tricky matrix for proteomics* - Ludovica Sofia GUADALUPI - Università degli Studi di Bari "Aldo Moro"

16:58 – **OR 7** – *Simultaneous determination of chlorinated and phosphorus organic pesticides and PAHs in honey samples for identifying anthropogenic impacts and related sources* - Sergio PASSARELLA - Università degli Studi del Molise

17:11 – **OR 8** – *PFAS extraction from water and wastewater using an off-line multi-SPE prototype equipment for high throughput and sensitivity LC-MS analysis* - Sara GRANAFEI - Servizi Chimici Ambientali s.r.l.

17:24 – **OR 9** – *Atmospheric Bulk Deposition of Polycyclic Aromatic Hydrocarbons in the City of Rome* - Giuseppe IANIRI – Istituto superioer di Sanità

17:40 – **POSTER SESSION & *Fcazz e Birr***
21:00 – **Social dinner**

Friday 26th May – session 1: Food & Proteomics

Moderatori: Dott.ssa Maria A. ACQUAVIA & Dott.ssa Mariachiara BIANCO

09:00 – **Keynote 2** – *QqQ-LIT mass spectrometry strategies for the identification of polyphenols in food matrices and waste product* - Eleonora OLIVA – Università degli Studi di Teramo

09:20 – **OR 10** – *Exploring the Metabolome of Pistachio Kernels and By-products through FT-ICR Mass Spectrometry* - Cinzia LELLA - Università degli Studi di Bari "Aldo Moro"

09:33 – **OR 11** – *Protein profiling and varietal comparison of lentils seeds and hulls by discovery proteomics* - Antonella LAMONACA - CNR-ISPA & Università degli Studi di Bari "Aldo Moro"

9:46 – **OR 12** – *Dispersive Liquid-Liquid Microextraction followed by GC-MS Analysis for Determining Phthalates Released in Preserved Meats* - Mariolina FANELLI - Università del Molise

9:59 – **OR 13** – *Determination of 2-dodecylcyclobutanone and 2-tetradecylcyclobutanone as markers in X-ray irradiated dairy products by HS-SPME/GC-MS analysis* - Andrea CHIAPPINELLI - Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata

10:12 – **OR 14** – *Untargeted metabolomic analysis of Parmiggiano Reggiano PDO using a multivariate curve resolution approach* - Samuele PELLACANI - Università degli Studi di Modena e Reggio Emilia

10:25 – **OR 15** – *Shotgun Proteomic Analysis of an Italian Chickpea Genotype* - Aldo LANZONI - Università di Catania

10:38 – **OR 16** – *Development of innovative methods for multi allergen quantification in processed foods* - Anna LUPARELLI- CNR-ISPA

10:51 – **OR 17** – *Enrichment of Phosphorylated Peptides in Insect Flour Using Molecularly Imprinted Polymers* - Simona RIGANTI - Università degli Studi di Bari "Aldo Moro"

11:05 – **11:35 Coffee Break**

Friday 26th – session: 2 Miscellaneous

Chairmen: Dott. Davide CONIGLIO & Dott.ssa Fabiola EUGELIO

11:35 – **Invited Speaker** – *Material screening by Inductively Coupled Plasma Mass Spectrometry for characterization of background in low radioactivity experiments* - Francesco FERELLA - INFN - LNGS

11:55 – **OR 18** – *Comparison of two validated methods for determination of L-dopa in Vicia faba L. and Phaseolus vulgaris L. beans* - Carmen TESORO - Università degli Studi della Basilicata

12:08 – **OR 19** – *Spatial mapping of multiple neurotransmitters in a model of cognitive impairment: MALDI-Mass Spectrometry Imaging as support to novel CB2 agonist discovery* - Emanuela SALVIATI - Università degli Studi di Salerno

12:21 – **OR 20** – *Application of a Nano Ultra-Performance Liquid Chromatography–High Resolution Mass Spectrometry platform for the characterization of the modification induced by small molecule inhibitors on the Lysines Acetylome profile of H3 and H4* - Manuela Giovanna BASILICATA - Università degli Studi di Salerno

12:34 – **OR 21** – *Development, validation and application to large cohort screening of a sub-5 min 4D Lipidomics approach* - Fabrizio MERCIAI - Università degli Studi di Salerno

12:47 – **OR 22** – *Bio-orthogonal non-canonical amino-acid tagging (BONCAT) of pancreatic ductal adenocarcinoma* - Asia BOTTO - Università di Pisa & Fondazione Pisana per la Scienza ONLUS

13:00 – **Greetings**

13:30 – **14:30** – **POSTER SESSION & lunch**

Scientific Program

Plenay Lecture

Region-specific lipid markers of plaque outcome revealed by mass spectrometry imaging of human carotid atherosclerotic plaques

Francesco Greco[1,2,3], Giulia Bertagna[4], Laura Quercioli[4], Angela Pucci[5], Silvia Rocchiccioli[6], Mauro Ferrari[4], Fabio A. Recchia[1], and Liam A. McDonnell[2]

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***Keywords**: Atherosclerosis plaque outcome, MALDI MSI, lipids*

Atherosclerotic plaque rupture can cause thrombi, occlusion of downstream vessels, and thereby adverse clinical events. Lipids constitute one of the main components of the lesion and are the mediators of many mechanisms involved in plaque progression and stability. The effect of different types of lipids on specific regions of the plaque could be one of the drivers of plaque destabilization. The effect of lipids on plaques have not been studied *ex-vivo*, and their investigation is complicated by the heterogeneity of atherosclerotic lesions. Here we report a matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI) investigation of the region-specific lipid composition of symptomatic and asymptomatic human carotid atherosclerotic plaques. Carotid atherosclerotic plaques were collected from 9 symptomatic and 20 asymptomatic patients. Plaques were analyzed by MALDI MSI of lipids and adjacent sections were analyzed by histology and immunofluorescence to segment the plaques into histomolecularly distinct regions. The MALDI MSI dataset was then co-registered to the segmented histological image and the lipid composition of the different regions from symptomatic and asymptomatic plaques were compared. Macrophage-rich regions from symptomatic lesions were found to be enriched in phosphatidylcholines (hypothesized to be synthesized to counteract excess free cholesterol [1]), while the same region from asymptomatic plaques were enriched in polyunsaturated cholesteryl esters and triglycerides, molecules characteristic of functional lipid droplets [2]. Vascular smooth muscle cells (VSMCs) of asymptomatic plaques were enriched in lysophosphatidylcholines and cholesteryl esters, know to promote VSMC proliferation and migration [3], crucial for the buildup of the fibrous cap stabilizing the plaque. We investigated with MALDI MSI the region-specific lipid composition of the atherosclerotic plaques from symptomatic and asymptomatic patients, in which the presence of symptoms such as transient ischemic attack were used as a proxy of plaque vulnerability. The analysis identified specific lipid markers of plaque outcome which could be clearly linked to known biological characteristics of stable plaques.

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Keynote Presentations

Mass spectrometry for the characterization of organic materials: from ancient to contemporary artworks

Elena C. L. Rigante[1], Michele Pellegrino[1], Vito Nettis[1], Tommaso R. I. Cataldi[1,2], and Cosima D. Calvano[1,2]

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***Keywords**: non-invasive sampling, pigments, binders*

Mass spectrometry (MS) is increasingly used for analytical chemical investigations in Cultural Heritage diagnostics to preserve and restore historically valuable objects. The binder (*i.e.*, medium) of the painting layer has been crucial since ancient times to produce a consistent, coloured mixture applied over the support and priming layer. Organic binders such as protein-based materials (*e.g.*, eggs, milk, and animal collagen), lipid-based materials like siccative oils, and polysaccharide-based natural gums were extensively used historically. However, natural binders have been replaced by synthetic ones, especially acrylic and alkydic polymeric resins, in tempera, spray paints, and varnishes since the 20th century [1]. Currently, there are numerous analytical techniques available for the chemical characterization of organic components in artworks, including various spectroscopies, gas and/or liquid chromatography (LC) coupled with MS. Soft ionization sources, such as matrix-assisted laser desorption ionization (MALDI) and electrospray ionization (ESI), are commonly used in this field due to their high sensitivity and their ability to analyze complex samples and challenging proteomics and lipidomics studies. The focus of this presentation is to provide an overview of soft ionization MS applications in the investigation of ancient and contemporary artworks, with a particular emphasis on the characterization of organic components (*e.g.*, binders, additives, and pigments) in antique sculptures, paintings, parchments, and modern street art murals. We will explore the potentialities and versatility of MALDI-MS and LC-ESI-MS applied to investigate a wide range of compounds, from peptides to organic pigments and polymers. We will also focus on a quasi-non-invasive sampling method for proteinaceous binders [2,3] following a bottom-up protocol for characterizing extracted proteins.

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QqQ-LIT mass spectrometry strategies for the identification of polyphenols in food matrices andwaste product

Eleonora Oliva[1], Fabiola Eugelio[1], Federico Fanti[1], Sara Palmieri[1], Eduardo Viteritti[1], Manuel Sergi[2], and Dario Compagnone[2]

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***Keywords**: Polyphenols, EPI, LC-LIT-QqQ*

Phenolic compounds (PCs) have biological role in plants, mainly related to antioxidant capacity [1], and it is closely linked to their chemical structure since they have many nucleophilic sites available for different reactions, such as: glycosylation, esterification, methylation, oxidation, *etc.* [2]. This leads to the formation of a large number of conjugated forms and hence to the challenge to develop analytical methods capable of providing a complete characterization of the samples, and to selectively identify a set of PCs belonging to a specific phenolic class. The wide range and different compounds depending on different conjugation and polymerization form. Nowadays an exhaustive identification of PCs realm is a challenging task in analytical chemistry field.

In this work, the potential of the hybrid linear ion trap – triple quadrupole mass spectrometry coupled with high-performance liquid chromatography (HPLC-LIT-QqQ) was exploited, in order to obtain more structural information for the putatively identification of PCs by the development of different approaches, capable of selective identification of the common moiety of interest. A selective determination by means of precursor ion scan (PI), neutral loss (NL), MS^3 (MS/MS/MS) [3], and enhanced product ion (EPI) [4] acquisition modes was achieved, based on the study of the PCs structures and their relative conjugated forms, such as phenolic acids esters, glycosidic derivatives of flavonoids. These approaches were tested and validated on different type of vegetal and food matrices led to the identification and evaluation of a wide range of PCs.

In our opinion, this approach represents a complete and useful tool for the determination of PCs in different matrices, as it can provide exhaustive information on the forms conjugated with specific molecules, allowing a rapid and effective putative identification for wide range of PCs derivates, such as mono-, di- and tri-glycosylated or other conjugates, without the need to employ a large number of standards.

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Invited Speker

Material screening by Inductively Coupled Plasma Mass Spectrometry for characterization of background in low radioactivity experiments

Francesco Ferella[1]

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***Keywords**: ICP-MS. Radioactivity, astroparticle physics*

Search for very rare astroparticle physics events, such as Neutrinoless Double Beta Decay or direct Dark Matter interactions demands for extremely low background environments and materials for the experiment construction. The former requirement is fulfilled by underground laboratories, such as the Laboratori Nazionali del Gran Sasso of INFN, where the overburden rock reduces the cosmic ray flux by six orders of magnitude with respect to the sea level. The latter requirement, *i.e.*, the material selection and cleaning for low-background experiment, increasingly challenges the international scientific community to develop new purification techniques and push the sensitivity to the edge of instrumental limits. Indeed, given the variety of materials used for the construction of experiments (*i.e.*, metals, plastics, electronics compounds, loaded liquids, pressurised gases) and the very low levels of radio contamination required, versatile and cutting-edge purification and characterisation techniques are needed.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) [1] is one of the most suitable technique for material screening because of its versatility and additional features such as high sensitivity (ppt level), short analysis time (days), small mass of samples (mg), possibility to acquire many elements simultaneously, and development of efficient analysis methods. Furthermore, several processes and chemical treatments are involved in the production of the raw materials, thus the effect of each step on the final product must be known in detail in order to be able to optimize the production protocol from the radiopurity point of view. ICP-MS is the only screening technique capable of investigating contaminations in all stages of production, for example separating bulk contaminations from surface ones by means of progressive chemical attacks.

In order to reach excellent results with ICP-MS, the workspace is crucial, because environmental contaminations must be avoided. For this reason, clean-rooms are ideal environment to perform both sample's treatment and characterization through ICP-MS [2].

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Oral Presentations

Mass spectrometric characterization of methyl carbamates of phosphatidylethanolamines and phosphatidylserines generated in the mitochondrial lipid extracts of mouse embryonic fibroblasts

Andrea Castellaneta[1], Vito Porcelli[2], Ilario Losito[1,3], Serena Barile[2], Alessandra Maresca[4], Valentina Del Dotto[5], Ludovica S. Guadalupi[1], Cosima D. Calvano[1,3], Valerio Carelli[4,5], Luigi Palmieri[2,6], and Tommaso R.I. Cataldi[1,3]

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***Keywords**: Phospholipid methyl carbamates, mitochondrial lipidomics, high-resolution tandem mass spectrometry*

The rapid expansion of mass spectrometry (MS)-based lipidomics has led to coping with the inherent challenges related to the sample preparation and analysis, which might impair the reliable identification and/or quantification of lipids in complex samples. In recent years, the hyphenation of hydrophilic interaction liquid chromatography (HILIC) with mass spectrometry (MS) has been successfully employed in our laboratory to unveil phospholipids artificially generated from undesired enzymatic side reactions occurring during sample preparation [1,2]. In this work, the presence of methyl carbamates of phosphatidylethanolamines (PE) and phosphatidylserines (PS) in the lipid extracts of mitochondria obtained from mouse embryonic fibroblasts was ascertained by HILIC with electrospray ionization single and multi-stage MS, performed using synergically a high resolution (quadrupole-Orbitrap) and a low resolution (linear ion trap) spectrometer.

There are two ways to make methyl carbamates of phospholipids. The first way involves a chemical reaction with phosgene and methanol, which can happen when extracting lipids using chloroform/methanol solutions. The second way might be represented by an enzymatic reaction that happens accidentally due to bacterial contamination, which was discovered during testing on a sample of murine mitochondria. A lipid extraction carried out using purposely photo-oxidized chloroform and deuterated methanol on standard PE and PS indicated that the first route was not significant in this case. Therefore, the methyl carbamates were likely to be formed through the enzymatic route caused by bacterial contamination. Recognizing this enzymatic route is important for understanding how methyl carbamates are generated in biological systems and how we can minimize their occurrence when they are an artifact.

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Mass spectrometry in urinary cotinine determination as a passive tobacco smoke biomarker

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***Keywords**: mass-spectrometry, cotinine, children*

Passive smoking is to be understood as involuntary inhalation by third parties of substances from the combustion of tobacco and it's responsible for a considerable share of childhood respiratory disease, including bronchial asthma and acute respiratory infections, but also for the risk of lung cancer and ischemic diseases in adults.

In humans, nicotine is mainly metabolized in the liver and in smaller amounts in the lung and brain, the main metabolite, excreted through the urine of the person, is cotinine [1].

Cotinine is the most representative metabolite, about 75% of nicotine, of which 10-15% is eliminated as such, 12-17% conjugate and the remaining part in other secondary metabolites.

Cotinine is a known biomarker of exposure to active tobacco smoke, and numerous papers correlate its urinary concentration with the number of cigarettes smoked, but it is interesting to evaluate if it is a good biomarker of exposure also to low levels of active tobacco smoke (few cigarettes) or of passive smoke. In order to studies this, we collected two spot urine samples from 568 healthy children, 6-11 years old, who live with a variable number of smokers and reside in two Italian regions with different levels of urbanization and industrialization.

LC-MS/MS determinations were carried out on a 4000 triple quadrupole mass spectrometer (Sciex), equipped with a TurboIonSprayTM interface. Chromatography was performed on an Atlantis dC18 column using variable proportions of 10 mM aqueous formic pH 3.75 acid and methanol at a flow-rate of 0.20 mL/min [2]; quantitative analyses were performed by method of additions in matrix using internal deuterated standard.

Urinary concentrations of cotinine in children who have smoker cohabitants are significantly higher than those in children who live with non-smokers, both in the samples collected in the evening and in the next morning, regardless of place of residence. Moreover, the urinary cotinine levels were significantly higher in urine samples collected in the evening than those collected in the morning. No relations between urinary cotinine levels and urbanization and industrialization grades were observed.

In addition, the urinary cotinine is confirmed as a good biomarker to secondhand smoke exposure since it positively correlates with the number of cigarettes smoked by cohabitants.

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BAG3 in glucose homeostasis: proteomics characterization of BAG3 knockout in pancreatic β-cells – un update

Federica Di Marco[1], Verena Damiani[1], Alessia Lamolinara[1], Maria C. Cufaro[1], Manuela Iezzi[1], Rossano Lattanzio[1], Michael Hahne[2], Maria C. Turco[3], Piero Del Boccio[1], Vincenzo De Laurenzi[1], and Damiana Pieragostino [1,3]

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***Keywords**: tissues; BAG3; proteomics*

The BAG3 protein, expressed in ß-pancreatic cells, regulates insulin secretion and the BAG3 silencing in mouse insulinoma cells decreases intracellular insulin content and increases insulin secretion in response to glucose stimulation. Our research group has previously observed how BAG3ßKO mice, used as a mouse model, show a condition like a hepatic steatosis. The proteomics workflow consists of tryptic digestion with FASP protocol of samples, nanoLC-Orbitrap-MS/MS analysis, label-free quantitative proteomics, evaluation of differential proteins and Functional Gene Ontology term enrichment analysis through IPA software. Proteomics analysis on the liver of BAG3ßKO mice (compared to WT) detects an increase in cholesterol synthesis and a decrease in the formation of ketone bodies. Functional proteomics analysis, using Ingenuity Pathway Analysis (IPA software), subsequently detected a shifted in metabolism towards lipogenesis with down-regulation in fatty acid ß-oxidation, ethanol degradation and oxidative phosphorylation in KO mice livers. The same mouse model was used to study the pathophysiological function of BAG3 in glucose homeostasis on the spleen (extra-hepatic organ) to identify and characterize any alterations due to its silencing in β cells of the pancreas. BAG3ßKO mice show impaired insulin sensitivity, secrete more insulin after glucose stimulation and become insulin resistant at 64 weeks of age. These mice also develop poorly defined amyloidosis in the spleen. Due to the difficulty of identifying and characterizing protein markers in amyloid plaques, we carried out a first proteomics analysis to study and describe the phenotype expressed in BAG3ßKO mice to confirm and describe the molecular composition of amyloid plaques. 49 proteins identified and quantified, appear to be associated with the amyloid database: in particular, Apolipoprotein-A2 (identified as exclusive presence in BAG3ßKO mice) regulates lipoprotein metabolism while C4 binding-protein interacts with the serum P component of amyloid. SAP appears to be upregulated in BAG3ßKO mice, which confirms the precipitation of SAP in amyloid plaques since it is downregulated in serum samples from mice at 64 weeks. From these preliminary data it can therefore be deduced how BAG3 in ß-pancreatic cells plays an important role in maintaining cell mass and function and its silencing can increase the tendency to develop insulin resistance and amyloidosis.

References

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Capillary Electrophoresis-Mass Spectrometry for Comprehensive Profiling of Free and Conjugated Estrogens

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The bioavailability and biological activity of estrogens is tightly regulated by phase I/II metabolic transformation processes associated with hydroxylation, glucuronidation or sulfation that are critical to human health development and fertility. However, there is lack of a specific, sensitive yet robust methodology for comprehensive estrogen profiling given the wide dynamic range and chemical diversity of endogenous/exogenous estrogen metabolites present in complex biological fluids, such as urine.

The application presented is one of the first reports of estrogen analysis by CE that allows for direct speciation of intact and highly polar estrogen conjugates with high selectivity and minimal sample handling. Qualitative identification of unknown steroid conjugates and their positional isomers can be realized by high mass accuracy TOF/MS in conjunction with prediction of ion migration behavior by CE that is relevant to applications in clinical chemistry, environmental analysis, and food science.

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LC/MS-MS assessment of CFTR Modulators Concentrations in Biological Fluids from Adult and Children with Cystic Fibrosis

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***Keywords**: cystic fibrosis transmembrane regulator (CFTR), drug monitoring, CFTR modulators*

Highly effective CFTR modulator therapies are transformational treatments for people with cystic fibrosis, who are gaining quality and quantity of life.

The new modulators of the cystic fibrosis transmembrane conductance regulator (CFTR), ivacaftor, elexacaftor and tezacaftor (ETI) are the first drugs that act directly on the underlying pathophysiological mechanism of cystic fibrosis (CF). However, there is a growing need of monitoring their concentrations in biological fluids in order to understand their long-term effects in patients, especially pregnancy and lactation [1,2].

Therefore, the aim of this study was to develop a new LC/MS-MS method for the quantification of elexacaftor (ELX), tezacaftor (TEZ), and ivacaftor (IVA) in fluids from a woman with CF who had a pregnancy in her late 30s while under treatment with ETI and child tissues by.

The method developed used a small sample volume (100 µL) of plasma of breast milk that was mixed with 4 volumes of methanol containing the internal standard lumacaftor (10 pg/µL) and extracted by protein precipitation. LC-MS/MS was performed on 10 µL of extracted samples for a total run time of 8 minutes. Standard curves and quality controls (QC) were obtained spiking a pool of blank plasma and/o milk and with analytes from different batches of working solutions of IVA, TEZ, and ELX. The 8-point calibration curve, ranging from 0.39 to 50 pg/µL, included the lower limit of quantitation (LLOQ).

The results indicated that IVA, ELX and TEZ were present at ng/mL levels in both maternal milk and plasma, as well as in the plasma of the child. Our results demonstrate that the methodology developed and employed here represents a step forward over previous published methods since it allows quantification of ETI from a small amount of biological fluids, which can minimize the impact of withdrawal procedures, and with a linear dynamic range of pg/mL,. Thus, it can be useful for real life, post-marketing monitoring of ETI in patients with CF, including pregnant women, mothers, and babies.

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Natural rubber: a tricky matrix for proteomics

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Keywords: *natural rubber, LC-ESI-MS/MS, proteomics*

Hevea brasiliensis is the primary source of natural rubber (NR) and the main raw material used in tyre manufacturing [1]. NR has a distinctive internal network structure that, when combined with non-rubber components, creates properties such as low thermogenesis and excellent durability during exhaustion cycling [2]. Proteins play a vital role as one of the main non-rubber components and serve as a critical branch point in the natural rubber network. A deeper understanding of these minor components could help modulate the mechanical and technological properties of finished tyres, resulting in high-performing products. The great challenge in protein analysis is signified by their low content (about 1.5-4%) and by their complex network interactions, which result in their very hard extraction processes from NR.

Whilst in-depth proteome analysis of latex extruded from *H. brasiliensis* has been reported [3], the proteins of NR obtained after the coagulation of latex have not been characterized yet. Our goal is to describe the proteome of NR by using an efficient protein extraction method and a bottom-up approach. This method involves using liquid chromatography coupled with mass spectrometry (LC-MS) to separate and identify peptides released from proteolysis before subjecting them to tandem MS for further characterization. We tested several extraction protocols on NR that had previously been treated by a cryo mill. The most performing protocol turns out to be a novel "one pot" one where a mix of reducing agent tris(2-carboxyethyl) phosphine, 2-chloroacetamide, sodium deoxycholate and ammonium bicarbonate was used alongside an ultrasonic homogenizer. The isolated proteins were digested by trypsin and subjected to LC-MS/MS analysis; the tandem mass spectra generated were interpreted through dedicated software and databases and manually validated. Two major proteins of NR were identified: the rubber elongation factor protein (REF) and the small rubber particle protein (SRPP). These proteins are critical for the fatigue crack resistance and longevity of tyres. Although there are minor proteins involved in the process as well, their extraction process is not yet optimized and their specific roles are still unclear [3]. Further research is needed to fully understand the complete biosynthesis process of NR and optimize the characterization of all involved proteins.

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Simultaneous determination of chlorinated and phosphorus organic pesticides and PAHs in honey samples for identifying anthropogenic impacts and related sources

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***Keywords**: honey, pollutant, anthropic impact*

The extensive use of pesticides around the world has been due to the growing need to meet the world demand for food. The use of such substances has enabled the control of insects and weeds that reduce production yields. Unfortunately, these substances have proven harmful to human health by causing oxidative stress, reduced male fertility and increased miscarriages in women. Furthermore, many of these substances are very thermodynamically stable and therefore remain in the environment, contaminating water, the ecosystem and thus the raw materials for foodstuffs. Honey, "the natural sweet substance that *Apis mellifera* produces from the nectar of plants", may contain these substances either due to environmental contamination, or due to the use of some of them inside the hive to control the presence of parasites harmful to bees. [1].

The aim of this work was to develop an analytical method for the simultaneous determination of pesticides and polycyclic aromatic hydrocarbons (PAHs) by means of ultrasound vortex assisted dispersive liquid-liquid microextraction (UVA-DLLME) followed by gas chromatography analysis coupled with ion trap mass spectrometry (GC-IT/MS). The proposed method is sensitive, reliable and reproducible. The compounds are extracted by 20 µL of a 1:1 mixture of heptane/toluene, the recoveries are between 80% and 109%. The limit of detection (LOD) is between 40 ng mL^{-1} and 180 ng mL^{-1}, the limit of quantification (LOQ) is between 100 ng mL^{-1} and 300 ng mL^{-1}.

Subsequently, the developed method was applied to real samples to assess the presence of pesticide residues in honey samples. The anthropogenic impact present in the different production areas was then assessed and the source of the PAHs was identified by means of the Fu/(Fu+Py) ratio in relationship to the Ant/(Ant+Phe) ratio [2].

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PFAS extraction from water and wastewater using an off-line multi-SPE prototype equipment for high throughput and sensitivity LC-MS analysis

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Keywords: *PFAS, multi-SPE extraction, LC-MS*

Per- and polyfluoroalkyl substances (PFAS) are a large class of synthetic chemicals that present numerous analytical challenges. As a result of the strong carbon fluorine (C-F) bond, PFAS chemicals have unique properties, such as high resistance to temperature, pH, oxidation, abrasion, high hydrophobicity and oil-repellent properties [1]. However, PFAS are not easily degradable, classified as persistent, bioaccumulative and toxic under the REACH Regulation. Since the 2000s the European Commission has taken restrictions and regulatory actions including sector-specific legislations for these compounds. Legislative Decree 18/2023 (implementation of Directive (EU) 2020/2184 of the European Parliament and of the Council) established the "PFAS sum" equal to 0.1 µg/L as the parameter value for the quality of drinking water analyzed by a method that guarantees a limit of quantification equal to 30% of the limit value. More restrictive is the limit value for the discharge of wastewater into surface water established by Piemonte regional Law 25/2021, according to which the limit of PFOS must be 0.02 µg/L until the 2026 and then 0.00065 µg/L.

Targeted analysis LC-MS direct injection methods are used to quantify PFAS in various matrices, however the direct injection does not satisfy the lowest quantification limits, so an off-line/on-line SPE extraction is usually required. The common off-line SPE method uses high sample volumes and organic solvents, needs longer evaporation steps and analysis time, however its advantages are greater method flexibility, less matrix effect in MS spectrometry, repeated measurements with the same extract and cheap equipment [2]. Conversely, on-line SPE uses a small volume, reduced analysis time and high throughput, minimal consumption of organic solvents, but less method flexibility, more matrix effects and expensive equipment [2]. A prototype of multi-SPE off-line extraction equipment was designed and developed by our laboratory with Lab Service Analytica srl in order to combine the best features of both SPE approaches, i.e. small sample and organic solvents volumes, flexibility, less matrix effect, automatic sample processing with high throughput. In 30 min per sample an extract is obtained ready for the subsequent LC-MS analysis without more concentration steps and in a fully automated way that can be controlled remotely. Preliminary recovery data on laboratory fortified sample matrix is in the range of 70-130% for nearly all native compounds and within the range of 50-200% for nearly all isotope dilution analogs. A limit of quantification equal to 0.004 µg/L for PFOS is guaranteed. Further optimizations of the SPE protocol are currently in progress.

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Atmospheric Bulk Deposition of Polycyclic Aromatic Hydrocarbons in the City of Rome

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***Keywords**: Atmospheric bulk depositions; PAHs; public health*

Population exposure to polycyclic aromatic hydrocarbons (PAHs) results not only from inhalation of airborne dust but also through ingestion of contaminated food (mainly vegetables) due to deposition of the settleable particulate material on which PAHs are bound [1]. In this regard, the European Union, through Directive 2004/107/CE urges member states to promote research on the effects of PAHs on human health from atmospheric deposition [2]. In this work, the deposition rate of settleable dust and major carcinogenic PAHs (Benz[a]anthracene, Chrisene, Cyclopenta(c,d)pyrene, Benzo[b+j+k]fluoranthenes, Benzo[a]pyrene and Indeno[1,2,3-cd]pyrene) in the urban area of Rome were measured monthly. The monitoring lasted for a full year, starting in March 2022 and ending in April 2023. Moreover, the monitoring was characterized by the collection of settleable dust at two different heights (vertical profile), one at street level and the other at a height of about twenty metres. The comparison of the deposition flows for the eight PAHs in the two locations shows higher average annual values at the location at street level (P1) than at the one positioned at a height of 20 meters (P2), with annual average values expressed as ΣPAHs in $ng \times m^{-2}d^{-1}$of 12.0 and 7.5, respectively. The annual averages of the deposition rates on the ground level of the total settleable particulate material (PM) measured in the P1 and P2 stations are respectively 51.7 and 18.7 $mg \times m^{-2}d^{-1}$. These values justify the higher levels of PAHs in the depositions collected at station P1 and highlight the greater contribution to the deposits made by the phenomena of dust rising from the ground.As regards the depositions of the eight PAHs in both locations there is an overall limited seasonal variability, recording the lowest average concentrations in summer (10.2 for P1 and 0.9 $ng \times m^{-2}d^{-1}$ for P2) and the highest ones in winter (17.1 for P1 and 2.4 $ng \times m^{-2}d^{-1}$ for P2). In Europe there is still no legislation that sets limit values relating to the deposition of settleable particulate material and PAHs bound to it. To carry out an evaluation, the data obtained can be compared with the reference concentrations indicated in the guidelines and regulations of other European countries. The deposition rates of the total settleable particulate material present values (51.7 and 18.7 $mg \times m^{-2}d^{-1}$) significantly lower than the reference limits of other countries such as for example in Germany (maximum 350 $mg \times m^{-2}d^{-1}$ as an annual average) and in the United Kingdom (200 $mg \times m^{-2}d^{-1}$). For PAHs, if the average annual concentration of Benzo[a]pyrene in the depositions measured in both locations is taken as a reference (1.6 for P1 and 0.5 $ng \times m^{-2}d^{-1}$ for P2) it can be concluded that the average annual deposition fluxes are in line with those measured in other Italian urban areas (*e.g.*, Val d'Agri in Basilicata region, 3.2-4.1 $ng \times m^{-2}d^{-1}$) and in Europe.

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Exploring the Metabolome of Pistachio Kernels and By-products through FT-ICR Mass Spectrometry

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***Keywords**: Pistachio, FT-ICR Mass Spectrometry, Green Chemistry*

In this work, the characterization of fatty acids, polyphenols, and plant flavonoids in kernel, shell, and hull from pistachio plant (*Pistachia Vera* L., variety Napoletana) was performed. While some papers have focused on lipidic and phenolic profile of kernel and hull [1], very limited information exists for shell. Since the exploration of natural resources such as plant by-products for the extraction of bioactive compounds is in line with the principles of green chemistry, which have the objective of developing sustainable and eco-friendly processes for the benefit of human health and the environment [3], we aimed at characterizing the metabolome profiles of by-products and comparing them with kernel. The three samples, kernel, hull and shell, were subjected to green extraction by water and ethanol and then analyzed by ultra-high-resolution mass SolariX XR 7-Tesla Fourier-Transform Ion Cyclotron-Resonance Mass Spectrometer (FT-ICR-MS, Bruker Daltonics, Bremen, Germany) in direct infusion mode. Fatty acids were also quantified by GC-MS. The ultra-high resolving power, sensitivity and mass accuracy of FT-ICR-MS allowed to identify metabolic networks in complex samples, such as plant extracts in a simple, rapid, and comprehensive way [2].

A total of 58 compounds were identified comparing annotated masses obtained in positive and negative ionization mode with specific databases. The chemical formula of a variety of compounds was assigned in samples: the hull showed the greatest number of exclusive formulas (1167 in positive and 1267 in negative ionization mode, respectively) while the kernel and the shell share a larger amount of formulas (348 in ESI^+ and 286 in ESI^-), even though the kernel, hull and shell are characterized by totally distinct textures, colors and organoleptic properties. Moreover, the number of compounds in common between the edible part and the two by-products of pistachio plant is extremely low: 292 molecules in ESI^+ and 349 in ESI^-. Van Krevelen and Kendrick diagrams were employed to describe the classes of compounds present, using an in-house software: lipids were identified as the most represented and abundant compounds in all the samples. Indeed, the hull is characterized by a large number of fatty acids, 16:0, 18:2 and 18:3. The shell also contains a large number of lipids and lipid-like molecules, including saturated fatty acids (18:0 and 16:0), while the kernel is the most enriched one in terms of unsaturated fatty acids (mainly 18:1). Further evidence of the hull's potential is given by its antioxidants content. These results will lead to new studies that aim to enhance pistachio plant wastes for the recovery of important nutraceutical compounds.

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Protein profiling and varietal comparison of lentils seeds and hulls by discovery proteomics

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Keywords: *lentil hulls; LC-HR-MS/MS; proteomics*

Among legumes, the lentil (*Lens culinaris*) is a major dietary component in many Mediterranean and Asian countries due to its high nutritional value, especially protein [1]. Italy is the eighth producer of legumes in Europe; however, about 20% of the total legume production is represented by organic waste deriving from the decortication process [2]. The valorization and recycling of these waste materials is particularly interesting in view of the concept of circular economy based on the principle "reduce, reuse, recycle".
In this investigation, we focused on two varieties of lentils (Lens Culinaris Medik), namely Eston green (EG) and Crimson red (CR), characterizing whole seeds and their by-product deriving from the decortication industrial process. Indeed, this by-product is rich in bioactive compounds and may be considered an eco-friendly source of health-promoting compounds [3]. The aim of this work was to characterize by high resolution mass spectrometry and advanced bioinformatics tools, the proteomic profile of EG and CR seeds and hulls in order to identify specific hulls proteins and potential differences between the two varieties, in the perspective to disclose potentiality as well as pitfalls of hulls reuse in derived commodities.
Seeds and hulls samples were extracted with denaturing buffer and subjected to 16h trypsin digestion after proper denaturation, reduction and alkylation steps. The LC-MS/MS analysis were carried out according to well established conditions for discovery analysis [5]. The raw data were processed by Proteome Discoverer v.3.0.1.27 (Thermo Fisher Scientific) for peptide/protein identification against a customized database restricted to Fabaceae taxonomy. Protein list was elaborated for protein classification according to their family/putative function. Most proteins have been identified and classified into 13 categories endowed with specific functions and properties: storage proteins, enzymes, Heat Shock Proteins, LEA Proteins, annexins, actins and others. From a qualitative point of view, these protein classes are common to the two varieties. From a quantitative point of view, the predominant class for the lentil hulls was the storage proteins (80%-85%): cupin (11S-legumins and 7S-vicilins) and prolamins (albumins and non-specific LTP). Confirmed and potential lentil allergens were identified according to official databases and very recent literature [1]. For both varieties, the most common allergens were Len c 1.0101, Convicillin and Len c 1.0102, well-known lentil allergens present in all plant allergen databases. As demonstrated, although lentil hulls are rich in beneficial compounds, the persistence of main allergenic proteins typical of seed cotyledons, raised safety concerns and requires proper attention by food manufacturer in case of reuse in derived commodities.

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Dispersive Liquid-Liquid Microextraction followed by GC-MS Analysis for Determining Phthalates Released in Preserved Meats

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Keywords: phthalates, packaging, GC-MS

In the field of packaging materials, the compounds that are added to the plastic material contributing to the gelling of polymeric materials, are the class of phthalates. These compounds improve the flexibility, elasticity, extensible and workability of plastic materials but, as a result of particular conditions occurring in the environment (thermal stress, obsolescence, etc.), can migrate from the packaging into the food. Even if they are detected at trace or ultra-trace concentrations, these compounds are harmful to human health [1].
The aim of this study is to develop a method to study the release of phthalates in these foods and cooked ham was chosen as the first matrix. The phthalates are extracted through the dispersive liquid-liquid microextraction (DLLME) technique whereas the analyses are carried out by gas-chromatography equipped with a mass spectrometry detector (GC-MS) [2]. The method developed in the present work was characterized by the choice of the best extraction solvent and the modification of the pH of the matrix. Among the different food matrices for daily consumption, we have focused our attention preserved meats, which are normally packaged in plastic packaging.
Good sensitivity and reproducibility of the method developed together with analytically significant recoveries with regard to phthalate molecules were obtained. It can be said that of all the tests carried out, there is a quantitatively significant recovery by using iso-octane as a solvent and working at pH 6. For example, DBP showed, out of an average of 4 daily trials, a recovery of about 97 %. The good recoveries obtained for phthalates do not correspond to a similar reasoning for bisphenol-A which did not present sufficiently adequate recoveries, probably related to the different molecular structure of BP-A compared to phthalate ones.
The results obtained from the analyses fall within the legal limits with regard to the release of phthalates. It remains to be clarified the suspected and high concentration of di-ethil-exil phthalate extracted from the matrix, which shows a concentration value of about 47 ppb, which can open up more or less different scenarios, so further studies are needed to deepen this information.

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Determination of 2-dodecylcyclobutanone and 2-tetradecylcyclobutanone as markers in X-ray irradiated dairy products by HS-SPME/GC-MS analysis

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Keywords: Dairy products, Food Irradiation, HS-SPME/GC-MS

Food irradiation is a clean and safe non-thermal technology used worldwide to improve safety, enhance and extend shelf-life of several foodstuffs such as fruits, vegetables, legumes, spices, meat, fish etc. [1-3]. To date, in Europe, screening and confirmatory validated or standardized protocols are available to identify whether or not food products have been treated with ionizing radiation [1]. Some procedures are chemical methods, based on the determination of specific radio-induced markers, as 2-Alkylcyclobutanones (2-ACBs), that are generated from radiolysis of triglycerides, exclusively by irradiation [1-2]. In particular, 2 dodecylcyclobutanone (2-DCB), which comes from palmitic acid (16:0) and the 2 tetradecylcyclobutanone (2-TCB), which comes from stearic acid (18:0), are the golden markers of irradiated lipid-containing foods [4]. The European reference method for determination of 2-DCB and 2-TCB, the EN 1785, is a time and solvent-consuming procedure [5]. In this work, an alternative, rapid, sensitive and selective method, based on headspace-solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME/GC-MS), previously validated for irradiated meats [2], was applied to determinate 2-DCB and 2-TCB in several X-ray irradiated dairy products, different in fat and moisture content. The two markers were identified in all types of irradiated samples, even if 2-TCB showed a lower sensibility than 2-DCB. Moreover, a linear response was observed in all the matrices studied for both these analytes, at increasing irradiation dose over the experimental range of 0.5–5.0 kGy. The minimum dose level and limit of detection in X-ray irradiated and spiked samples, were 0.5 kGy and 5.0 μg L-1, respectively. Furthermore, in all matrices and at all irradiation doses tested, the proportionality between 2-DCB and 2-TCB was proved. Finally, chemometric analysis showed how the extraction of two investigated analytes differs in relation to the characteristics of dairy product samples. Hence, 2-DCB and 2-TCB could also be identifiers of the different technological processes and ripening. This method results an efficient and reliable tool to monitor a wide number of dairy products and it could be used for food safety control plans.

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Untargeted metabolomic analysis of Parmigiano Reggiano PDO using a multivariate curve resolution approach

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Keywords: metabolomics, ROI-MCR, food authentication

In this study, the Region of Interest-Multivariate Curve Resolution (ROI-MCR) method is proposed for the analysis of non-target metabolomics data [1]. The case of Parmigiano Reggiano PDO certification "Prodotto di Montagna Progetto Territorio" [2] is investigated due to its role in supporting the sustainability of mountain areas and offering local economic opportunities. Cheese samples were analyzed using UHPLC-HRMS with an untargeted approach to characterize their compositional profile. The complex metabolomics big datasets generated by UHPLC-HRMS were analyzed using the ROI-MCR strategy, involving the Region of Interest (ROI) procedure for data filtering, compression, preprocessing, and storage steps. Multivariate Curve Resolution-Alternating Least Square (MCR-ALS) was then applied to the previously MS ROI preprocessed datasets to resolve the elution profiles and spectra fingerprints of the chemical constituents. The resolved features were putatively identified using different reference spectral libraries. Peak areas of the elution profiles of the chemical constituents resolved by the combined ROI-MCR procedure were analyzed using Principal Component Analysis (PCA). Parmigiano Reggiano "Prodotto di Montagna Progetto Territorio" samples were found to be well-differentiated from other cheese samples in terms of amino acids and oligopeptides content, which are the markers showing the highest discrimination potential. The study presents a promising approach for the analysis of non-target metabolomics data using ROI-MCR, which may have significant implications for food quality in the future.

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Shotgun Proteomic Analysis of an Italian Chickpea Genotype

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Keywords: *New psychoactive substances, Synthetic Cannabinoids, Magnetic solid phase extraction*

Legumes represent one of the most important sources of human food after cereals [1]. Particularly, chickpea (*Cicer arietinum* L.) is one of the third most important legume crop in the world, following the common bean and the pea. Up to date, comprehensive studies on agronomic and genetic influences on chickpea protein composition are lacking. Therefore, the aim of the present project is the development of a "shotgun" proteomics approach [2] to investigate the qualitative and quantitative changes in the protein composition of the chickpea genotype "Pascià" grown under different environmental and agronomic conditions. The preliminary results allowed the identification of about 900 proteins in the genotype "Pascià" grown under rainfed conditions and using the following fertilization conditions: 40 kg/ha N. This approach will be extended to different chickpea genotypes in order to understand the influence of genetic variability, environmental conditions, and cultivation conditions on chickpea protein fraction composition.

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Development of innovative methods for multi allergen quantification in processed foods

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***Keywords**: food allergy; hidden allergens; UHPLC-HRMS/MS analysis*

The growing global incidence of allergy to nuts and peanuts has constantly increased the need of new strategies to protect sensitive consumers. Nowadays, the best choice to avoid unawares immunological reactions still remains the total removal of the culprit products from the diet. However, traces of nuts and peanuts could contaminate other foods, especially processed products, as consequence of cross-contamination events occurring during production. In the light of this, the development of a multi-target method based on mass spectrometry for the detection of traces of allergens could represent a useful tool to protect allergenic individuals also preventing the removal of safe food from their diet.
In this work, an ultra-high-performance liquid chromatography coupled with high resolution Orbitrap™-based mass spectrometry (UHPLC-HRMS/MS), for the simultaneous detection of five nuts species (almonds, hazelnuts, walnuts, cashews, and pistachios) and peanuts in an incurred bakery product (cookie) is described.
Experiments were accomplished on an incurred cookie model food produced in-house by adding roasted and powdered nuts/peanut upon dough preparation and before baking. The following pipeline was used for the LC-MS/MS method development: i) LC-HRMS/MS untargeted analysis of both raw and roasted ingredients for the selection of the most appropriate candidate marker peptides for detecting nuts and peanut allergens, ii) constraining the resulting peptides according to the specific criteria previously reported in literature [1], iii) assessing of the impact of processing on the detection of candidate peptides in cookie extract, iv) selection of the most appropriate marker peptides for tracing nuts/peanut in cookie food, v) evaluation of method performance by building up specific curve calibration for each investigated peptide and assessment of the most relevant method parameters (linearity, limit of detection (LOD), limit of quantification (LOQ), repeatability inter- and intra-day), vi) comparison of the method performance with the VITAL 3.0 thresholds. Suitable and thermostable peptide markers for each allergenic ingredient were selected and the LOD and LOQ values obtained for each allergen calculated in incurred cookies (referred to the protein content) allowed to detect levels of contamination complying with the reference thresholds set for each allergen and recommended (action level 1) by the VITAL program v 3.0.
Additionally, method precision provided good results for all the allergenic ingredients analyzed in this matrix [2]. This method could represent an important tool to support the risk assessment for allergen management also preventing the overuse of PAL (Precautionary Allergen Labelling).

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Enrichment of Phosphorylated Peptides in Insect Flour Using Molecularly Imprinted Polymers

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***Keywords**: molecular imprinted polymer, phosphopeptides, novel food*

The scarcity of food resources and limited availability of arable land and fresh water has led to the need to investigate alternative protein sources. As the population continues to grow, finding alternatives to animal proteins is essential to bridge the gap between food supply and demand. For this reason, in recent decades new foods known as "novel foods" have also been introduced into the Western diet [1]. These include proteins from aquaculture or insect meals, which have high potential as alternative sources in the human diet. Novel foods are those new foods, novel ingredients, or novel preparation techniques, regulated by EU food legislation under Regulation (EC) 258/97, for which there is not a "significant" consumption as of May 15, 1997, within the European Union (EU), the date when the regulation came into force.

This study focused on the characterization of the protein profile of insect flours to identify their possible post-translational modifications such as phosphorylation [2], which has never been described so far. A bottom-up proteomics approach was used to identify the proteins present in *Tenebrio molitor* flour. After testing different protein extraction protocols, the tryptic digestion was performed in solution. The digests were analyzed by reversed-phase liquid chromatography (RPLC) coupled with electrospray mass spectrometry (ESI), and tandem mass spectrometry (MS/MS). The analysis was conducted before and after a phosphopeptide-specific enrichment procedure based on the use of molecularly imprinted polymers (MIPs) [3, 4]. The whole procedure was initially assessed on a training matrix sample, such as milk, where phosphorylated caseins are known to be present and thus applied to T. molitor flour proteins. RPLC-ESI-MS/MS spectra were acquired both on native and MIP enriched samples allowing to retrieve great differences in the type and numbers of peptides. Indeed, the collection of tandem mass spectra together with bioinformatic data post-processing enabled the recognition of 14 phosphorylated peptides in milk samples and 23 phosphopeptides in insect flour after MIP enrichment. To confirm the occurrence of post-translational modification the enriched samples were subjected to an additional digestion with alkaline phosphatase to allow the dephosphorylation.

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Comparison of two validated methods for determination of L-dopa in *Vicia faba* L. and *Phaseolus vulgaris* L. beans

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***Keywords**: validation, tandem mass spectrometry, chromatography separation*

Two validated methods were compared for the determination of L-dopa in *Vicia faba* L. and *Phaseolus vulgaris* L. beans: LC-UV and LC-MS^2, with recovery values at three concentration levels ranging from 83 to 117%, [1,2]. Reversed phase high performance liquid chromatography (RP-HPLC) coupled to DAD and Q-Trap detection are used. For the first method a Discovery C18, 250*4,6 mm, 5 µm analytical column and an isocratic elution with a mobile phase consisting of formic acid 0.2% and methanol and a flow rate of 1 mL/min were used [1]. For the LC-MS^2 method the optimal conditions were obtained by using a Kinetex C18, 100*2.1 mm, 2.6 µm analytical column and an isocratic elution with a mobile phase consisting of formic acid 0.2% and methanol and a flow rate of 0.2 mL/min [2]. Both methods adequately resolve L-dopa within a 10 min run time. Due to the low molecular weight and the occurrence of the chromophore group, the LC-UV method is more suitable than LC-MS^2, especially for medium/high analyte content plant matrices, showing detection and quantification limits of 41.4 and 45.2 µg/L, respectively; on the other hand, lower limits of detection of 0.4 ng/mL and quantification of 1.1 ng/mL were reached with the LC-MS2 method. The two techniques have been fully validated according to EURACHEM and ICH guidelines and proved to be reproducible and sensitive [3, 4]. Both methods can be successfully applied for the analysis and quantification of L-Dopa content in various legumes. Quantifying L-dopa in *Vicia faba* L. and *Phaseolus vulgaris* L. beans allows to evaluate their potential use as natural adjuvants to the pharmacological treatment of Parkinson's.

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Spatial mapping of multiple neurotransmitters in a model of cognitive impairment: MALDI-Mass Spectrometry Imaging as support to novel CB2 agonist discovery

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***Keywords**: MALDI-Mass spectrometry imaging, Memory impairment, Neurotransmitters*

Mass Spectrometry Imaging (MSI)-based spatial-omics can be a valid analytical technique to investigate the correlation between various neuromodulators and their dynamic spatial distribution, with the aim to understand their involvement in neurological disease and to develop focused and effective treatments.
In the present study, the neuroprotective role of a novel designed and synthesized agonist of endocannabinoid receptor CB2 in a scopolamine-induced amnesia mouse model has been investigated.
Specifically, MALDI Mass Spectrometry Imaging (MSI) has been employed to explore the localization and modulation of neuromediators in the context of brain structures critical for memory and cognition, related to their interconnection with CB2 stimulation [1-3].
Different neurotransmitters, directly or indirectly involved in memory and cognitive processes, including γ-aminobutyric acid (GABA), glutamate (Glu), serotonin (5-HT) and acetylcholine (ACh) were simultaneously mapped by using a derivatization with a reactive matrix (FMP-10), across different brain regions of mouse brain tissue sections from two coronal levels by the combination of two MSI strategies: MALDI-FT-ICR and MALDI-TOF MSI.
The selective stimulation of CB2 receptors with the agonist revealed a significant restoring of GABA, Glu, 5-HT and Ach levels in key brain regions for memory and cognition, such as the hippocampus, Infralimbic and Lateral Orbital cortex, which were dysregulated by the acute administration of scopolamine.
These results underline the potential of MALDI-MSI-based as a useful strategy to explore the neuro-pharmacodynamic effects of CB2 stimulation in cognitive impairments, supporting the development of novel small molecules as well as potential diagnostic and prognostic tool.

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Application of a Nano Ultra-Performance Liquid Chromatography–High Resolution Mass Spectrometry platform for the characterization of the modification induced by small molecule inhibitors on the Lysines Acetylome profile of H3 and H4

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***Keywords**: Histone post-translational modifications, Mass spectrometry, Bottom-up*

Histones regulate chromatin structure and gene transcription through their diverse post-translational modifications (PTMs). Histone PTMs impact on various important cellular phenotypes and their aberrant regulation is linked to a wide variety of diseases, including cancer [1]. Accurate quantification of PTMs is an ongoing challenge due to the variety of modifications and their wide dynamic range of abundance. Liquid chromatography coupled to mass spectrometry (LC-MS) has become the most suitable method to analyze histones and histone PTMs in a large-scale manner. Lysine acetylation in histones is a dynamic process that involves a balance between the actions of two classes of enzyme: histone acetyltransferases and histone deacetylases. In this study, we investigated the ability of some thiobarbiturates derivatives to modulate the histone acetylation levels in human colon cancer cells (HCT-116). For this purpose, we applied a label free bottom-up proteomics approach to identify and quantify main PTMs of histones H3 and H4. HCT-116 cells were incubated for 6 hours with investigated compounds, and the isolated histones were digested with trypsin. However, use of trypsin on histones results in peptides that are too short to retain on reversed phase chromatography columns and a lack of charge density, making them not amenable for MS. Thus, propionic anhydride derivatization of free amine groups on the N termini and lysines before a trypsin cleavage was applied. Finally, samples were desalted using C18 and HyperSep™ SpinTip and analyzed using a nano-HPLC coupled online with an Orbitrap Fusion MS. NanoLC was configured with a 75 μm ID × 25 cm × 2.6 μm biozenTM Peptide XB-C18 (SL) nano-column using an EASY-nanoLC nanoHPLC. Data were acquired using a data-independent (DIA) acquisition method, consisting of a full scan MS spectrum performed in the Orbitrap at 120,000 resolution, followed by 16 MS/MS with windows of 50 m/z using HCD fragmentation and detected in the ion trap. DIA data were searched using EpiProfile 2.0 [2]. Our results showed that thiobarbiturate derivatives reduced, even if with different potency, the acetylation level of H3K9, H3K14, H3K18, H3K23 and H4K16. Interestingly, all these modifications are mostly modulated by KAT3B (p300).

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Development, validation, and application to large cohort screening of a sub-5 min 4D Lipidomics approach

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***Keywords**: Trapped ion mobility, Lipidomics, High throughput*

Reversed-phase UHPLC-HRMS represents the golden standard in untargeted lipidomics. Conventional methods are characterized by long analysis times that are not suitable for large cohort screening, thus coverage and gradient length represent the short blanket of these approaches. Ion mobility mass spectrometry has emerged as an attractive tool to perform lipid profiling [1], being an additional dimension of separation.
In this contribution we evaluated the implementation of trapped ion mobility mass spectrometry (TIMS) to reduce analysis time while keeping accuracy and coverage.
Starting from popular untargeted conditions, such as charged surface hybrid stationary phases (CSH) and 20 minutes length gradients, the whole analysis time was scaled down by reducing column (5 cm) and gradient length [2].
Taking advantage of ion mobility dimension and parallel accumulation–serial fragmentation (PASEF) operation mode, cleaner MS/MS spectra and resolution of co-eluting compounds were obtained.
The platform was initially tested against human plasma extract, and, following extensive manual curation, was able to annotate, and relatively quantify with confidence 392 lipids, belonging to 24 different subclasses, ranging from pmol/mL to µmol/mL in 4 min of analysis time, reporting average MS/MS scores, Δppm and ΔCCS errors of: 876.5, -0.57 and 1.47 respectively. To prove the robustness of the method, the same workflow was repeated after two months delivering satisfactory results.
The method was finally applied to profile the plasma lipidome differences in a cohort of mothers with congenital heart disease in offspring (157 patients) compared to controls (162 patients). Multivariate data analysis revealed the modulation of numerous lipid sub-classes showing the usefulness of this approach in biomedical or drug-discovery applications to rapidly profile plasma lipidome of large cohort groups and extract reliable valuable biological information.

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Bio-orthogonal non-canonical amino-acid tagging (BONCAT) of pancreatic ductal adenocarcinoma

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***Keywords**: Proteomics, Mass spectrometry, pancreatic cancer*

Pancreatic ductal adenocarcinoma (PDAC) has a very poor prognosis, with less than 10% of patients alive five years after diagnosis. The prognosis of cancer patients is much improved through earlier detection. A recent study reported 5- and 10-year survival rates of 49% and 31%, respectively, for T1-2, N0, and R0 tumors [1]. Earlier diagnosis would enable treatment to begin when the tumor is in its earlier stages, and so improve patient prognosis. This aim would be achieved by developing a method able to isolate and identify characteristic markers of PDAC.

PDAC is characterized by a robust fibroinflammatory response, widespread vascular collapse, and hypoperfusion that make the tumor highly hypoxic and nutrient deprived. This microenvironment promotes tumor invasion, and progression leads to chemotherapy or radiotherapy resistance and eventual mortality.

To isolate newly synthesized protein from hypoxic cells we developed and optimize a protein labeling approach that is cell-type/phenotype-specific, and enables the solation through the labeled proteins. Bio-orthogonal non-canonical amino-acid tagging (BONCAT) is a technique that labels proteins with an azido moiety.

The azido moiety is introduced during protein synthesis using methionine surrogates, then with a simple click chemistry reaction, only the proteins that contain the azido moiety will be enriched [2].

Cell-specific BONCAT labeling can be achieved using azidonorleucine (ANL) as the Met surrogate. ANL is only incorporated into proteins by a specific mutant form of the protein methionyl-tRNA synthetase (MetRS*). Accordingly, ANL labeling can be restricted to cells equipped with this mutant form. The DNA of MetRS* is introduced into cells by transduction, using specific promoters to target specific cell phenotypes (*e.g*,. HIF1α for hypoxia). Once ANL has been added to the media all methionines in the newly synthesized proteins from MetRS* transfected cells will be substituted for ANL.

Here we will show progress in method development, including results showing the incorporation of BONCAT labels into PDAC tumor cells and their enrichment by optimization of the click chemistry reaction.

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Poster Presentations

Magnetic Solid Phase Extraction: a new method for the determination of synthetic cannabinoids in hair

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Keywords: *New psychoactive substances - Synthetic Cannabinoids – Magnetic solid phase extraction*

New psychoactive substances (NPS) are substances often known as either designer or synthetic drugs, or by the more popular term of 'legal highs'. They are analogues of existing controlled drugs and pharmaceutical products or newly synthesised chemicals, created to mimic the actions and psychoactive effects of licensed medicines and other controlled substances.
Synthetic cannabinoids (SC) first formally identified and reported to the EMCDDA in 2008, initially being used as alternatives to herbal cannabis, particularly to avoid detection in those settings with forensic drug testing regimes. To date, SC represent the largest and most structurally diverse class of NPS [2]. A fast and inexpensive detection of these class of drugs in biological samples is of utmost importance to monitor their diffusion since SC have proliferated worldwide in many different structures, forms and potencies. The choice of the matrix to be investigated depends on the purpose of the analysis. Hair testing has gained increasing attention since it is a unique material for the retrospective detection of drugs, due to its large detection window. The extraction of SC from the inner of the hair structure is a critical point as well as the clean-up of hair extracts. Different strategies have been proposed for this purpose. Hair incubation is often performed by digestion of the hair matrix with NaOH; or by the incubation of hair with methanol or other solvents for several (4 to 18) hours. Clean-up is crucial in both cases and usually can be performed by classic liquid-liquid extraction (LLE) or solid phase extraction (SPE). The main issue is the lack of versatility of all these techniques if compared with the breadth of analytes that should be considered. In recent years magnetic-solid phase extraction (MSPE), which is based on the use of functionalised magnetic nanoparticles for dispersive SPE, is gaining interest in various fields, but its application in forensic and toxicological field is currently underdeveloped.
The aim of this study is the development of a new protocol for qualitative and quantitative determination of several SC in hair.
Drug extraction from hair was performed by means of MeOH incubation followed by a MSPE clean-up. Magnetic particles were synthesized according to Yu et al. [3], with a slight modification, and functionalized with a C18 shell. Particles shape and functionalization was characterised by means of FT-IR, TEM and SEM analyses.
Preliminary results showed good recoveries for all selected analytes and a remarkable clean-up efficiency. The protocol has been validated following national and international guidelines.

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Sterols in the offshoots of globe artichokes: an investigation by liquid chromatography and Fourier-transform mass spectrometry with atmospheric pressure chemical ionization

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Keywords: *RPLC-APCI-FTMS, plant sterols, globe artichoke's offshoots*

Plant sterols (PS) are secondary metabolites that can be found in vegetables in various forms including free sterols, esters of fatty acids, sterol glycosides, and acyl sterol glycosides. [1]. These compounds have been reported to have nutraceutical properties, being able to reduce blood cholesterol levels and exert anti-obesity, anti-diabetic and anti-inflammatory activities [2]. It is worth noting that sterols can be found also in plant-derived products that are not typically considered for their nutritional properties. These sterols often have potential health benefits for humans. This is the case of offshoots obtained from globe artichoke (*Cynara cardunculus* L. subsp. *scolymus*), that, despite being included in the List of Traditional Agri-Food Products of Apulia (Southern Italy), are often considered as by-products and discarded.

In the present study, an investigation on major sterols occurring in the offshoots of globe artichokes of two Apulian genotypes ('Lucera' and 'Locale di Mola') will be described. Sterols were identified by reversed-phase liquid chromatography (RPLC) coupled to high-resolution Fourier-transform mass spectrometry with atmospheric pressure chemical ionization (RPLC-APCI-FTMS). The proposed analytical method revealed the presence of several PS in the sample extracts obtained from 'Lucera' and 'Locale di Mola' offshoots of globe artichokes. Besides major plant sterols, such as stigmasterol and β-sitosterol, the occurrence of campesterol, brassicasterol, and lanosterol was ascertained, thus increasing the number of species recently identified in similar matrices using NMR spectroscopy [3]. The extracted ion current (EIC) chromatograms obtained from RPLC-APCI(+)-MS data, referred to $[M\text{-}H_2O+H]^+$ ions of sterols, revealed the presence of several isomeric forms that deserve further investigation. Stigmasterol and β-sitosterol were quantified using commercial standards with an external calibration, and they were found to have higher levels in the offshoots of globe artichokes compared to other commonly consumed vegetables like tomatoes and cauliflower. This suggests that the offshoots of globe artichokes could be an excellent source of phytosterols in diets that include fruits and vegetables.

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Analysis of volatile compounds of dried fruit wastes by HS/SPME- GC/MS

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Keywords: *Food by-products, Volatile organic compounds, HS-SPME*

Agro-industrial by-products and wastes have caught special attention from the scientific community for being an available, cost-effective and sustainable source of a wide range of bioactive compounds. By-products and wastes are generated copiously by agroindustries, from fruits to marine products and, among them, fruit wastes are the most studied since possess diverse nutraceutical profiles [1].
Wastes from fruits and vegetables can also be used to prolong food shelf life [2, 3].
There is an important relationship between the presence of volatile organic compounds in food and multisensory flavour perception and therefore the acceptability of a product [4].
Headspace solid phase microextraction (HS-SPME) technique coupled with gas chromatography mass (GC-MS) is the main technique used to extract, isolate and enrich the volatile fraction from the sample matrix [5].
In this study, of the volatile organic compounds (VOCs) profiles from grounded dried fruit wastes (*e.g.*, skin, seeds, pulp) have been investigated and compared.
The extraction of VOCs has been carried out by SPME fibres (CAR/PDMS and PDMS/DVB) used at the same appropriate analytical conditions.
VOCs were analysed using a 6890N gas chromatograph (Little Falls, DE, USA) coupled with an Agilent 5975 mass selective detector, equipped with a Gerstel MPS autosampler (Gerstel, Baltimore, MD, USA). Differences in VOCs profile between the different parts (skin, pulp and seeds) and types of fruit (pomegranate and prickly pear) were evaluated.

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Innovative analytical tools for the detection of New Psychoactive Substances by high-resolution mass spectrometry

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***Keywords**: High Resolution Mass Spectrometry; NPS; Statistical Analysis*

The purpose of this work was to create a UHPLC-HRMS analytical platform for target and untarget determination of new and classic substances of abuse in seizures. The main difficulty in recognizing New Psychoactive Substances (NPS) lies in their dynamic nature, in the continuous synthesis and introduction on the market of new drugs, with similar but not identical structures to existing ones. These new drugs are not identified by classical data analysis software because they are not yet in the databases, but they may be potentially identifiable with predictive models, taking into account the similar fragmentation patterns.

In this study, the analytical determination was conducted by ultrahigh-performance liquid chromatography coupled to high-resolution mass spectrometry by exploiting a spectrometer Synapt G2-Si HDMS (Waters, Milford, MA, USA). Initially exploiting 57 analytical standards belonging to different classes of chemicals, chromatographic and mass spectrometry acquisition parameters were defined. The first analyses were carried out in full scan mode, to obtain retention times for each analyte, and later in MS/MS mode, with reasoned collision energy values, chosen in relation to the chemical characteristics of the precursor ion molecule, in order to obtain fragmentation spectra. Subsequently, to allow untargeted analysis, a chemometric model that would highlight the similarities between the chemical characteristic of each analyte was done. Specifically, a data matrix was created containing all values of *m/z* related to the fragments with their respective intensities, normalized to the intensity of the ion precursor. A principal component analysis (PCA) was then performed to obtain a chemometric model that would highlight common fragments within the various classes; also the PCA lays the foundation for creating a model that can predict the class of an unknown substance based on its fragmentation pattern. PCA was performed using MatLab, in total 19,614 accurate masses rounded to the third decimal place. With further analysis, the starting data can be expanded, and a model can be obtained that can characterize more substances and highlight the fragments they have in common. It will be possible, in this way, to take important steps forward in the analysis and recognition of New Psychoactive Substances.

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Cell-Surface Proteomics of Pancreatic Ductal Adenocarcinoma Cells and Exosomes

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***Keywords**: bottom-up proteomics, nHPLC-MS/MS, early diagnosis*

The study of membrane proteins is of particular interest in clinical research, since they are involved in many biological processes such as cellular communication [1]. Their location on the cell surface makes them attractive targets for pharmaceutical intervention, indeed they represent the majority of targets of pharmaceutical drugs [2].

This work is focused on the analysis of PDAC (Pancreatic Ductal Adenocarcinoma) cells and exosomes. PDAC is the most common pancreatic cancer. It has a five-year survival rate below 10% due to the fact that is usually diagnosed at an advanced stage when the available therapies are largely ineffective. It has been estimated that this type of neoplasm could become the second leading cause of death in Western countries [3]. The investigation of PDAC cell surface proteins can lead to the identification of new drug targets, and the identification of exosome surface proteins specific to PDAC, could enable the development of new biomarker assays for earlier diagnosis.

The hydrophobicity of membrane proteins and the embedding of the transmembrane domains within the phospholipid bilayer of the membrane means their extraction is more difficult with respect to the total cellular proteome. For this reason, specific methods of extraction and identification are frequently employed.

The aim of this work was the optimization of a method for cell surface proteomics. The method consists in the use of a cell-impermeable biotinylation agent that chemically derivatizes the lysines of cell-surface proteins. At the end of the reaction and after cell lysis, a neutravidin resin is used to isolation the biotinylated proteins. Finally, biotinylated denatured proteins are converted into peptides after proteolysis using trypsin and bottom-up proteomics analysis performed.

This technique has been applied to the PDAC cell lines SUIT 2-028 and PANC 1 and their exosomes.

Cell surface biotinylation is well established and the materials needed are readily available. The large number of cells typically used (~ 10^7 cells) [4] makes it difficult to apply to exosomes. The optimization was focused on decreasing the number of cells used in the analysis, so that the method could be applied to exosome preparations.

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Development and validation of a suitable method for the determination of secondary metabolite of THC

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Keywords: *Hair, UPLC-MS/MS, THC-COOH*

Cannabis Sativa L. is a plant species of Cannabis. In addition to its recreational use as a drug of abuse, the plant has widespread alternative uses including the production of food, cosmetics (hemp), textiles and medicinal applications [1]. In the early 1980s a series of studies began on the possibility of determining substances of abuse in the keratin matrix depending on their lipophilicity, molecular weight molecular weight, pKa and steric bulk in a stable manner over time. The analysis of substances of abuse in keratin matrix is an ideal addendum to the analysis of blood or urine analysis. When toxicology laboratories are required to investigate past exposure to cannabis, analysis of hair can provide powerful evidence. In particular, the target compounds in hair analysis are: Δ(9)-tetrahydrocannabinol (THC), the main psychoactive active compound of cannabis, the metabolite 11-nor-9-carboxy-Δ9-THC (THC-COOH) [2]. Usually, after ingestion, traces of substances and their metabolites are deposited into hair via the bloodstream and into the growing follicle. Quality assurance is a major issue in drug testing in hair resulting in new recommendations, validation procedures or inter-laboratory comparisons. Furthermore, recent trends in research concerning hair analysis are discussed due to the low concentration of legislative limit of THC-COOH (2pg/mg) as well as novel analytical procedures. The aim of this work focuses on basic aspects of method development and validation of hair testing procedures, in particular a SPE clean-up protocol was developed following Standard Practices for Method Validation in Forensic Toxicology (SWGTOX) guideline, in order to eliminate possible interfering compound and avoid the matrix effect in sample analysis considering the necessary enrichment factor to reach low limit of quantification.

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Development of an UPLC-MS/MS method for the evaluation of quinolizidine alkaloids profile in lupins

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Keywords: *alkaloids, lupins, UPLC-MS/MS*

Quinolizidine alkaloids (C_5NC_4 skeleton) are L-lysine derived compounds, having one or more nitrogen atoms usually contained in a heterocyclic ring system, which can be divided in bicyclic, tricyclic, and tetracyclic alkaloids [1].

These compounds are especially present in plants belonging to the *Lupinus* L. genus (Fabaceae family) and acting as a defense mechanism against pathogens and herbivorous animals. There are almost 70 different quinolizidine alkaloids found in various lupin species, which levels and combinations vary according to botanical and geographic origin, but also to soil composition and climate; they give bitter taste to the seeds and can cause even symptoms of poisoning in humans, affecting the nervous, circulatory and digestive systems. For this reason, bitter lupin seeds are not suitable for human or animal consumption without a proper debittering process [2]. According to this, the Regulations in Australia, New Zealand, France and Great Britain require compliance with a maximum level of 200 mg Kg^{-1} of alkaloids in lupin flours and in the seeds themselves [3].

There are few methods in the literature about the quantification of alkaloids in this specific food matrix; most of them reported the use of gas chromatography coupled to mass spectrometry (GC-MS), but recently there has been an increase of methods by ultra performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) to detect alkaloid compounds in various matrices, both food and biological [2]. In this work, a sensitive method involving the use of UPLC-MS/MS was developed in targeted mode, with the aim of the simultaneous quantification and determination of different alkaloids. An efficient extraction procedure followed by a suitable clean-up step was also developed by Solid Phase Extraction, in order to decrease the amount of interfering compounds and to obtain reliable recoveries [4]. The presented method was fully validated following FDA guidelines and then applied to different batches of raw *Lupinus albus* L. samples originating from Abruzzo region, varying in size and farming treatments.

According to the analysis results, the different sizes and farming conditions appeared to have a significant influence on the alkaloids content in lupin samples; in particular, the less grown lupins showed a significant major concentration of alkaloids, as well as the biological farming samples showed an increased amount of alkaloids, due to the nitrogenate fertilizers. This information can be helpful for a farmer to understand what type of seeds should be selected for human consumption and, consequently, for the most appropriate debittering process.

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Validation of a confirmatory analytical method for the determination of pesticides in fish samples by GC-MS/MS

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***Keywords**: Fish samples, Pesticides, Validation*

Pesticides cause ecosystem pollution so that they can be detected in the environment, particularly aquatic areas and coastal zones where they can induce adverse effects on living organisms [1]. As a consequence of this concern for human health, effective analytical methods are needed in order to assure food safety. Extraction is the first step of analytical procedures applied to the determination of organic pollutants residues in environmental matrices and living organisms. The Quick, Easy, Cheap, Rugged and Safe (QuEChERS) method developed in 2003 by Anastassiades et al. [2] for the analysis of pesticides in fruits and vegetables is an interesting extraction technique for complex biological matrices like fish. This method can be divided in two steps: a soft extraction with acetonitrile and a "clean up" by "dispersive solid phase extraction". The QuEChERS technique constitutes an easy method which also requires the use of a small quantity of solvent [3].
This study proposes the validation of an efficient analytical method for the extraction of pesticides in muscle of fishes using QuEChERS extraction, prior to detection and quantification by GC-MS/MS analysis. The matrix extract was used for calibration in order to compensate for matrix effects. PCB 209 was used as internal standard. This validation study produced good results in terms of precision, recovery rates, ruggedness, specificity, instrument linearity, LODs e LOQs, resulting in compliance with the most important validation guidelines. In particular, method linearity was verified using Matrix-matched calibration curves, in the range 2.5 - 250 µg/kg, calculating parameters such as determination coefficient (R^2), slope and intercept significance. The compliance with Regulation SANTE 11312/2021 [4] was verified, with recovery % in the range 80 – 120% and CV% < 20. Method selectivity was verified by analysing 20 samples of fish, different for lipid content, confirming the absence of interfering compounds. Finally, measurement uncertainty was calculated at 25.0 µg/kg for each analyte, confirming the applicability of this analytical method for routinely control activity of pesticide residues in fish samples.

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An in vivo gas chromatography-mass spectrometry evaluationof silicone oil contaminants and their possible correlation with retinal detachment

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***Keywords**: Box-Wilson Central Composite Design, silicone oil, gas chromatography-mass spectrometry, retinal detachment*

Most of the silicone oils used clinically in vitreoretinal surgery are composed of poly-dimethyl-siloxane.Sudden central visual loss in vitrectomized eyes soon after the removal of the silicone oil tamponade isan unexplained severe complication of this surgical procedure with incidence ranging from 1 to 30% [1]. Low-molecular weight components are the most likely responsible of a toxic effect on the retina leading to the visual loss [2]. In this study, for the first time, an in vivo assessment of contaminants in the 4 different types of viscous tamponade potentially responsible for retinal toxicity was performed. Firstly, an untargeted GC-MS approach was applied to identify the contaminants present in each tamponade used: both linear and cyclic siloxanes were identified in all the samples, being cyclic siloxanes already present in some of the commercial preparations. Then, a Box-Wilson Central Composite Design (CCD) followed by the multicriteria method of desirability functions [3] was applied to search for the optimal conditions for the headspace gas chromatography-mass spectrometry (HS-GC-(SIM)-MS) quantitation of the identified siloxanes. Extraction temperature (50-100 °C) and extraction time (20-60 min) were selected as main factors. A global desirability D=0,91 was calculated in correspondence to an extraction temperature and an extraction time of 100 °C and 20 min, respectively. The HS-GC-(SIM)-MS method was validated according to the bioanalytical method validation guidelines [4] and applied for the analysis of 42 patients categorized by the different type of tamponade used. Traces of hexamethylcyclotrisiloxane and octamethylcyclotetrasiloxane were observed in high percentages in all the groups examined. The correlation between the ocular concentration of siloxanes and the time of permanence in the eye may support a progressive passage of the substances into the retina.

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Multi-residues pesticides analysis in honey samples by high resolution mass spectrometry

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***Keywords**: Honey, Pesticides, UHPLC-Q-Orbitrap-MS*

In recent years, the demand for honey has increased because of its nutritional value, therapeutic effect and unique flavor. Thus, honey is widely consumed as medicine and its contamination may represent a serious health hazard. In particular, the presence of pesticide residues can affect physicochemical properties of honey and cause risks for consumers [1]. In this study an analytical methodology was developed to identify 44 pesticides in honey samples. A QuEChERS (quick, easy, cheap, effective, rugged, and safe) procedure was used for sample extraction and clean-up [2]. Ultrahigh-performance liquid chromatography electrospray Q-Orbitrap mass spectrometry (UHPLC/ESI Q-Orbitrap) was used for the analysis of samples in order to identify multiclass pesticides [3]. A complete chromatographic separation of 44 residues was carried out in 16 minutes. Ionization and fragmentation settings were optimized by analyzing pesticide standard solutions. In order to acquire high abundant fragments for the target compounds, the stepped normalized collision energy (NCE) of some precursors was optimized. Matrix-matched calibration curves, obtained using 0.5, 3, 10, 50 and 100 ng mL^{-1} standard solutions of chemical analogues, showed correlation coefficients ≥0.99 for all compounds. In our work, a fast, simple and reliable method based on QuEChERS extraction and cleanup-strategy, followed by UHPLC-Q-Orbitrap MS for multi-residues analysis in honey samples was established for an adequate determination of 44 pesticides. Two different energy NCE, i.e., 20 and 65 allowed the fragmentation of different precursors at their best efficiency. A total of 44 target analytes, including organophosphorus, organochlorines, phenylpyrazoles, pyrethroids and other pesticides, were successfully identified. Furthermore, other pesticide residues will be integrated using this valid analytical method in order to extend the application to different food matrices. The results of this work can be used for food safety control plans according to European legislation.

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Early-stage formation of dicarboxylic acids in mock-up samples investigated by RPLC-ESI-MS

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Keywords: *cultural heritage; LC-ESI-MS; dicarboxylic acids*

Oil painting is an ancient artistic technique, with the earliest known artwork found in a cave in Afghanistan dating back to the 7th century AD [1]. The process of oil painting spread to Europe in the 13th century, and today we have a better understanding of how to properly prepare and apply the paint binder, as well as the chemical processes that occur as the painting layers are exposed to environmental oxygen [2,3]. However, these works of art are susceptible to ageing and degradation, which can alter their appearance or even threaten their structural integrity [4]. To prevent such degradations, it is important to screen for characteristics that are correlated with the onset of these processes. One such characteristic is the formation of free dicarboxylic acids (FDAs), which are a product of the oxidation of drying oils. Detecting FDAs can serve as an early warning sign of deterioration in oil paintings. Currently, FDAs are mostly investigated using gas-chromatography coupled with mass spectrometry (GC/MS), which requires time-consuming sample preparations, including derivatization reactions [5]. To eliminate the need for such processes, a new approach based on reversed-phase liquid chromatography with MS by electrospray ionization in negative ion mode (RPLC-ESI$^-$-MS) was developed to monitor the occurrence and variation of FDAs in two model systems. These models were created by applying onto a glass slide a mixture of linseed oil and an inorganic pigment, such as HgS or $PbCrO_4$, following traditional recipes [6]. Agreement of retention time with standard compounds and tandem MS experiments confirmed the chemical identification of FDAs, containing 8, 9, 10, and 11 carbon atoms (*i.e.*, suberic, azelaic, sebacic, and undecanedioic acids, respectively). Our approach used micro-sampling with approximately 0.5 mg of the specimen and proved to be sensitive enough to detect an increase in the content of FDAs in our mock-ups over time (up to two years), which varied depending on the inorganic pigment used. In this communication, we will present the most significant findings of our study.

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Identification of spirulina allergenic proteins through liquid chromatography and mass spectrometry by marker peptides

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Keywords: *marker peptides, spirulina allergens, mass spectrometry*

The consumption of alternative protein sources, such as microalgae and insects, has become increasingly important in mitigating environmental pollution caused by animal protein production. However, it is equally important to evaluate the allergenic potential of these novel foods known for their high content of proteins (exceeding 50% of weight), along with vitamins, polyunsaturated fatty acids (such as ω-3 and ω-6), bioactive peptides and so on [1]. Spirulina, in particular, has been associated with allergic reactions in some individuals. Petrus et al. [2] identified the β-chain phycocyanin C protein as the reason for anaphylaxis in a 14-year-old teenager. This was determined through Western blot analysis on spirulina protein extracts and MALDI-MS analysis of tryptic digests from IgE-reactive fractions. To address this issue, we developed an innovative strategy, based on WHO/FAO guidelines, to identify potential allergenic proteins in microalgae [3]. In this study, we identified and quantified known and putative allergenic proteins in spirulina-based food supplements and processed food products [4] using high-resolution tandem mass spectrometry coupled with reversed-phase liquid chromatography and electrospray ionization. Three marker peptides were identified for the C-phycocyanin beta subunit, a known allergenic protein in spirulina. These peptides, chosen as qualifiers, ETYLALGTPGSSVAVGVGK and YVTYAVFAGDASVLEDR and as a quantifier ITSNASTIVSNAAR, were evaluated for their chemical and thermal stability in various foodstuffs. The method was validated for linearity, limits of detection/quantification, recovery, repeatability, reproducibility, matrix, and processing effects. Our findings provide insight into the potential allergenicity of spirulina-based products and can aid in the development of safer and more reliable food products for consumers.

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Development of an HPLC-MS/MS method for monitoring healthcare personnel exposed to antineoplastic drugs

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Keywords: *antineoplastic compound, HPLC-MS/MS, workers health and safety*

Antineoplastic compounds are drugs used in cancer therapy since they limit the growth and reproduction of tumor cells. However, their lack specificity is represented by numerous side effects. While the benefits of using these drugs outweigh the risks for patients with cancer, healthy individuals should avoid exposure, or at the very least, contamination should be minimized as reasonably achievable (ALARA principle - As low as reasonably achievable) [1].

The number of workers potentially exposed to these drugs is significant, including pharmacists, nursing staff, physicians, environmental services staff, research laboratory personnel. Exposure can occurs through dermal absorption and inhalation, but also from hand-to-mouth contact, accidental injection through a needle, and through the excretions and secretions (urine, feces, and sweat) of patients treated with these drugs [2].

To this end, an HPLC-MS/MS method has been developed to detect and quantify four of the most commonly used drugs (Gemcitabine, 5-Fluorouracil, Capecitabine, and Paclitaxel) in wipes and pads (as requested by a hospital). Wipes are gauzes rubbed on the surfaces to be sampled, while pads are placed in different anatomical areas of the operator for monitoring purposes. The aim of improving the wipe and pad sampling procedure has been achieved through the use of pre-packaged Alcohol Pads, ensuring a greater reproducibility but also practicality and speed of sampling.

Considering the hazardous nature of the analytes being sought, it was necessary to make the pretreatment step as simple, rapid, and manipulation-free as possible to avoid exposing the analytical chemist to risks. So, the method involves the extraction of the analytes from the pads in a single step by means of an organic solvent.

The method was applied to real samples (pads and wipes). The obtained data showed positive results in some of the analyzed samples. These samples confirm the existence of contamination risks. To minimize exposure, it is possible to pay closer attention during manipulation and preparation operations, wear personal protective equipment (PPE), and implement appropriate cleaning procedures.

In conclusion, it can be stated that the developed method features a simple sampling process, rapid pretreatment, and identification using the HPLC-ESI-MS/MS system, which harnesses the versatility of liquid chromatography and the sensitivity and specificity of mass spectrometry. To date, with the aim of demonstrate the workers exposure, the analysis of biological matrices, in particular oral fluid, is still in progress.

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