



MASSA
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MASSA 2026

Chieti, 22-25 June 2026

Book of Abstracts

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Agenda

DAY 1 – Monday, June 22

MASSA 2026 Scientific Program



Time	Speaker / Event	Affiliation / Chairs	Title
12:00	Registration		
14:30	Welcome and greetings		
	Session 1 – Nutrition and Active Compounds Chairs: Paola Montoro / Antonio Recchiuti		
14:40	PL.01. Luciano Di Martino	Maiella National Park – Maiella Geopark	Maiella National Park – Maiella Geopark Wilderness in the heart of the Mediterranean Sea
15:30	K.01. Adriano Rutz	ETH Zurich	Open Tools, Broad Knowledge: Empowering Researchers for Contextualized Metabolite Annotation
16:00	OR.01-S1. Tiziana Nardin	Fondazione Edmund Mach	Comprehensive UHPLC–HRMS workflow for nitrosamine profiling in meat products: formation mechanisms and dietary implications
16:20	OR.02-S1. Davide Corinti	Sapienza University of Rome	IR ion spectroscopy and mass spectrometry reveal surprising Zn(II)-mediated naringenin reactivity
16:40	Coffee break		
	Session 1 – Nutrition and Active Compounds Chairs: Emanuela Gregori / Roberto Larcher		
17:10	OR.03-S1. Antonella Lamonaca	Institute of Sciences of Food Production, ISPA-CNR	Disclosing the role of wholemeal semolina and drying conditions on the protein profile and digestibility of durum wheat pasta by HR-MS/MS
17:30	OR.04-S1. Mathieu Merlet	Institut Agricole Régional	Comprehensive phenolic profiling of <i>Reynoutria × bohemica</i> via LC-ESI-MS: valorizing invasive biomass
17:50	OR.05-S1. Stefania Pagliari	University of Milano-Bicocca	Chemical characterization and biological activity of <i>Myrtus communis</i> L. leaves: development of a NADES-UAE extract
18:10	OR.06-S1. Silvia Pianezze	Fondazione Edmund Mach	Isotopic application of high-resolution Orbitrap mass spectrometry: a case study on curcumin
18:30	End of the session		
19:00	Welcome party		

DAY 2 – Tuesday, June 23

MASSA 2026 Scientific Program



Time	Speaker / Event	Affiliation / Chairs	Title
	Session 2 – Mass Spectrometry for Human Health Chairs: Linda Monaci / Marco Gaspari		
09:00	K.02. Pierluigi Mauri	Institute of Technologies for Biomedicine	Clinical proteomics – from diagnosis to therapy
09:30	OR.07-S2. Claudia Fumagalli	University of Milano-Bicocca	An optimized IM-MS workflow for the analysis of native N-glycans from human plasma
09:50	OR.08-S2. Natalia Shelly Porto	University of Milano-Bicocca	Uncovering early renal alterations in Fabry disease through spatial proteomics
10:10	OR.09-S2. Matteo Mucci	“G. d’Annunzio” University of Chieti-Pescara	A multimatrix LC-MS/MS approach reveals airway exposure-response relationships for elxacaftor/tezacaftor/ivacaftor in cystic fibrosis
10:30	OR.10-S2. Viola Tintori	University of Milano-Bicocca	Single-Step DIA-PASEF Unlocks Comprehensive Antigen Profiling in Membranous Nephropathy from Minimal Microdissected Glomeruli
10:50	OR.11-S2. Vito Nettis	University “Aldo Moro” of Bari	The role of in-source fragmentation patterns in the RPLC–HRMS-based profiling of oxysterols in pancreatic tumour cell models
11:10	Coffee break and poster session		
	Session 2 – Mass Spectrometry for Human Health Chairs: Matteo Mucci / Flaminia Vincenti		
12:00	OR.12-S2. Anna Luparelli	Institute of Biomembranes, Bioenergetics and Molecular Biotechnologies, National Research Council (IBIOM-CNR), Bari	Development of a green LC-MS/MS method for the detection of peanut allergens in oil samples
12:20	OR.13-S2. Virginia De Vito	PharmaNutra S.p.A.	Zebrafish model for Sucrosomial® iron studies
12:40	OR.14-S2. Ilenia Bracaglia	Sapienza University of Rome	Untargeted metabolomics via UPLC-HRMS and ASCA-based statistical modelling to characterize metabolic alterations following acute and repeated bupropion exposure
13:00	Lunch		
	Session 3 – Environmental Applications Chairs: Cecilia Bergamini / Chiara Maccari		
14:30	K.03. Marcello Locatelli	“G. d’Annunzio” University of Chieti-Pescara	Mass spectrometry trends based on sustainability, environmental impact, and new tools for assessing adherence to green analytical chemistry principles

Time	Speaker / Event	Affiliation / Chairs	Title
15:00	OR.15-S3. Maria Assunta Crescenzi	University of Salerno	High-resolution mass spectrometry reveals the effects of water stress on olive xylem chemistry and spittlebug feeding
15:20	OR.16-S3. Michela Torrelli	Istituto Nazionale di Ricerca Metrologica (INRIM), University of Turin	An integrated quantification in soil from legacy to ultra-short chain PFAS: a multi-platform mass spectrometry strategy optimized via design of experiments
15:40	OR.17-S3. Serena Fiorito	“G. d’Annunzio” University of Chieti-Pescara	Comprehensive GC-MS profiling of glandular secretions in wild wolves (<i>Canis lupus</i>): characterization of volatile and semi-volatile semiochemicals
16:00	OR.18-S3. Enrico Davoli	SRA Instruments	Direct classification of Essential Oils with a Miniature, Deployable Mass Spectrometer Equipped with a Cold Plasma Ion Source
16:20	BI1. Lorenzo Zingaro	Agilent Technologies	Enhancing Sensitivity in Fluorotelomer Analysis with Hybrid Multisampler and High-End TQ-MS
16:30	Tea break and poster session		
	Session 3 – Environmental Applications Chairs: Anna Giulia Ruggieri / Francesco De Angelis		
17:00	OR.19-S3. Fabiola Eugelio	University of Teramo	Determination of pendimethalin metabolites in zebrafish using in silico prediction coupled with high-resolution mass spectrometry
17:20	OR.20-S3. Emanuele Ceccon	Restek Italy	PFAS: an analytical emergency finally solved?
17:40	OR.21-S3. Daniele Zatta	University of Padua	Validation of an enhanced GC-MS isotope dilution method for PFAS determination in water
18:00	DSM meeting		
21:00	Social dinner		

DAY 3 – Wednesday, June 24

MASSA 2026 Scientific Program



Time	Speaker / Event	Affiliation / Chairs	Title
	Session 4 – Omics in Clinical Research Chairs: Andrew Smith / Carmela Zacometti		
09:00	PL.02. Jens Soltwisch	University of Münster	Integrating MALDI-MS Imaging and Fluorescence Microscopy for Spatial Multi-omics at the Cellular and Subcellular Level
09:50	OR.22-S4. Greta Bindi	University of Milano-Bicocca	Linking lipid metabolism to the immune landscape of clear cell renal cell carcinoma through MS-driven spatial multi-omics
10:10	OR.23-S4. Alice Di Sebastiano	“G. d’Annunzio” University of Chieti-Pescara	Proteomic characterization of CAR-T cell-derived extracellular vesicles as a potential cell-free strategy for cancer immunotherapy
10:30	OR.24-S4. Nicole Monza	University of Milano-Bicocca	Advancing thyroid nodule diagnosis: integrating MALDI-MSI for detailed characterization of indeterminate lesions
10:50	Coffee break and poster session		
	Session 5 – Mass Spectrometry in Omics Sciences Chairs: Donatella Caruso / Gianluca Giorgi		
11:30	OR.25-S5. Federica Di Marco	“G. d’Annunzio” University of Chieti-Pescara	Phospho-proteomic profiling of Calu3 cells infected with SARS-CoV-2 reveals BCL2-associated athanogene 3 (BAG3) phosphorylation and AKT/MAPK signaling activation
11:50	OR.26-S5. Andrea Castellaneta	University “Aldo Moro” of Bari	In-source fragmentation of oxysterols: from analytical pitfalls to confident identification by high-resolution mass spectrometry
12:10	OR.27-S5. Sara Palmieri	University of Teramo	Untargeted UHPLC-HRMS metabolomics of water kefir: effect of different substrates on metabolic profiles
12:30	OR.28-S5. Alessandra Tata	Istituto Zooprofilattico Sperimentale delle Venezie	Breaking the 6-second barrier: evaluating the innovative DBDI-MS tool for rapid foodomics analysis
12:50	OR.29-S5. Mauro Paolini	Fondazione Edmund Mach	Monitoring of reactive carbonyl compounds in food matrices via SPME-GC-MS/MS
13:10	Lunch		
14:00	Departure for social trip		
20:00	Return to Chieti		

DAY 4 – Thursday, June 25

MASSA 2026 Scientific Program



Time	Speaker / Event	Affiliation / Chairs	Title
	Session 6 – Advanced MS Applications Chairs: Giuliana Bianco / Luciano Navarini		
09:00	K.04. Paolo Della Sala	University of Salerno	High-resolution mass spectrometry in supramolecular chemistry: an essential tool for characterizing host@guest complexes and self-assembled architectures
	Session 6 – Advanced MS Applications		
09:30	OR.30-S6. Sara Ghirardi	University of Milano-Bicocca	Mapping drought-driven metabolic remodelling in coffee fine roots by MALDI-MS imaging
09:50	OR.31-S6. Lorenzo D’Ippolito	Sapienza University of Rome	Formation of copper(II) oxide nanoparticles by ESI microdroplets deposition
10:10	OR.32-S6. Chiara Salvitti	Sapienza University of Rome	The curious case of TEMPO-photocatalysed Knoevenagel condensation: unveiling hidden reaction pathways through MS monitoring
10:30	BI2. Alessandro Fabiani	PerkinElmer	QSight 500 ultimate robustness for dirty matrices - unique source design for reduced sample preparation.
10:40	BI3. Daniela Peroni	SRA Instruments	Soluzioni per l’analisi diretta in aria di VOC, PFAS e metalli. Introduzione alla tecnologia ToFwerk
10:50	Coffee break		
	Session 7 – Advanced Data Analysis in Mass Spectrometry Chairs: Maria Assunta Acquavia / Piero Del Boccio		
11:30	OR.33-S7. Francesca Monzillo	University of Salerno	A multi-platform untargeted LC-HRMS workflow reveals metabolic differences between <i>Brassica oleracea</i> microgreens and vegetative plants
11:50	OR.34-S7. Francesco Bartolini	Sapienza University of Rome	Advanced data mining for oxylipin characterization by LC-HRMS
12:10	OR.35-S7. Alice Vetrano	University of L’Aquila	Protein secondary structure patterns in short-range cross-link atlas
12:30	OR.36-S7. Vasco Coelho	University of Milano-Bicocca	Co-registration of mass spectrometry and optical whole-slide histology images for computational pathology
	Award Ceremony Chairs: Antonio Recchiuti / Paola Montoro		
12:50	OR.37. Maria Concetta Cufaro	“G. d’Annunzio” University of Chieti-Pescara	FACS-Proteomics strategy toward extracellular vesicles single-phenotype characterization in biological fluids: exploring the role of leukocyte-derived EVs in multiple sclerosis
13:30	Closing and greetings		

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Plenary Lectures

Maiella National Park – Maiella Geopark. Wilderness in the heart of the Mediterranean Sea

Luciano Di Martino¹

¹*Maiella National Park – Maiella Geopark, via Badia 28, 67039 Sulmona (AQ), Italy*

Keywords: *Maiella, national park, geopark, biodiversity, geodiversity, conservation, heritage*

The Maiella National Park was established by Law No. 394 of December 6, 1991, and opened to the public by Presidential Decree of June 5, 1995. The park extends over 74,095 hectares in the green heart of Abruzzo, encompassing the provinces of Pescara, Aquila, and Chieti, as well as 39 municipalities. The park's institutional responsibilities include protecting natural biodiversity and the cultural heritage of local communities. The promotion of short agri-food supply chains is accompanied by the local development of the agricultural, livestock, and agro-energy sectors: revitalizing the primary sector is essential to maintaining the bond that unites farmers and shepherds with the local landscape. The rediscovery of popular traditions and the dissemination of culture, along with active protection of natural and semi-natural features such as waterfalls and berries, are the essential drivers of the Apennine rebirth for sustainable tourism, capable of expressing the uniqueness of the territory in synergy with contemporary socio-economic reality.

A National Park with over 25 years of history, supporting nature and local communities.

On April 22, 2021, the Maiella National Park was recognized as a UNESCO Global Geopark, joining the Global Geopark Network (GGN). This achievement attests to its geological significance, as well as its biodiversity, traditions, and historical, artistic, archaeological, and cultural heritage.

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2. DI MARTINO L., STANISCI A., VITALE D., DE CHIRO M. (EDS.). Turismo, Biodiversità, Heritage: dall'integrazione delle risorse alla progettazione territoriali. Edizioni Parco Nazionale della Maiella – Caramanico Terme 2018. ISBN 9788894189346

Integrating MALDI-MS Imaging and Fluorescence Microscopy for Spatial Multi-omics at the Cellular and Subcellular Level

Jens Soltwisch¹, Alexander Potthoff¹, Jan Schwenzfeier¹, Sebastian Bessler¹, Klaus Dreisewerd¹

¹ Institute of Hygiene, University of Münster, 48149 Münster, Germany

Keywords: MALDI-MSI, transmission Mode, MALDI-2, multi-omics, lipids, metabolites, single-cell analysis

The spatial analysis of lipid and metabolic profiles by matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) can add an additional important layer of molecular information to the growing field of spatial biology. This, however, requires increased sensitivity, robust measurements at cellular resolution, and a seamless integration of MSI data with other “omics” methods such as immunohistochemistry (IHC). A successful combination with (immuno-) fluorescence microscopy, for example, can provide complementary data on segmentation and identification of individual cells and their specific tissue microenvironments on the one hand and untargeted lipid and metabolite information of the same cells, on the other. Combining both modalities with the required accuracy, however, requires precise co-registration between optical and MS-derived molecular information while maintaining efficient and reproducible experimental workflows.

Here, we present a dedicated MALDI-2-MSI platform that provides a pixel size of 1 μm x 1 μm and below using a transmission mode geometry (t-MALDI). At the same time the setup integrates brightfield and fluorescence slide scanning microscopy directly into the t-MALDI ion source [1]. This way, it unifies optical imaging and MSI into a tightly coupled acquisition strategy and demonstrates how the tight conjunction of IHC and MALDI-MSI can enable molecular analysis on the level of specifically identified cells in situ.

The capabilities of the integrated t-MALDI-2 and IHC workflow are demonstrated with selected biological applications. Next to a description of technical details as well as improvements in sample preparation and data analysis, applications include the analysis of adaptive lipid profiles of infiltrating immune cells. Demonstrated on infected mouse bladder tissue as well as in a mouse tumor model, fluorescence-identified immune cell populations can be selectively targeted for subsequent t-MALDI MSI analysis, revealing distinct lipid profiles and spatially dependent molecular heterogeneity at the single-cell level. The in-situ character of analysis allows for the placement of lipid phenotypes in their respective microenvironments of the tissue and the direct correlation with antigen derived as well as mass spectrometry based phenotypical differences. In these examples, the employed strategy enables the investigation of the alteration and adaptation of molecular signatures during immune cell infiltration. Next to tissue analysis at the cellular level, the combined t-MALDI-MSI and optical analysis also allows for a first glimpse into the analysis of metabolic activity at the sub-cellular level on the example of phagocytosis in a cell culture model for macrophages.

Project PNRR IR “Strengthening BBMRI.it - Rafforzamento dell’Infrastruttura di Ricerca delle Biobanche e delle Risorse Biomolecolari in Italia”

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1. Potthoff et al.; Nat Commun., 16 (2025), 9129 DOI:10.1038/s41467-025-64603-8

Keynotes

Open Tools, Broad Knowledge: Empowering Researchers for Contextualized Metabolite Annotation

Rutz Adriano¹, Zamboni Nicola¹

¹ *Institute of Molecular Systems Biology, ETH Zurich, Zurich, Switzerland*

Keywords: *metabolite annotation, mass spectrometry, computational metabolomics, natural products research, open science*

Spectral library matching has long been the backbone of MS-based molecular annotation, yet it captures only a fraction of the evidence available by disregarding taxonomic plausibility, predicted fragmentation, cross-dataset occurrence, and the collective output of decades of natural products research. The challenge is making that broader corpus easily accessible to any researcher, not just those with the infrastructure or expertise to assemble it themselves.

Open tools now make this possible. Spectral libraries, in silico annotation engines, and knowledge graphs such as the LOTUS initiative [1], aggregating hundreds of thousands of organism–chemical associations, can be queried and combined within transparent, modular annotation workflows. Rather than replacing library matching, these resources act as orthogonal evidence layers that together sharpen candidate ranking and surface results that no single source would reveal [2]. The annotations produced are not opaque scores but traceable assignments that researchers can inspect and challenge.

Crucially, this model is participatory: every researcher who deposits their finding back into the shared resources strengthens the prior for the entire community. Open tools lower the barrier to both consuming and contributing knowledge, turning routine annotation into a collective act of scientific curation.

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2. Rutz et al.; Frontiers in Plant Science 10 (2019), DOI: 10.3389/fpls.2019.01329

Clinical Proteomics – from Diagnosis to Therapy

Davide Perico¹, Raffaello Viganò¹, Pier Luigi Mauri^{1,2}

¹ *Institute of Technologies for Biomedicine – ITB-CNR, Via F.lli Cervi 93, 20054 Segrate (MI), Italy*

² *Institute of Endotypes in Oncology, Metabolism and Immunology – IEOMI-CNR, Via Pansini, 5, 80131 Napoli*
All authors are part of Elixir Proteomics Community

Keywords: *Proteomics, Translational Medicine, Systems Biology, Mass Spectrometry*

Proteomics has become a valuable method for describing the molecular signature of diseases, providing insight into protein profiles contributing to disease severity and treatment outcomes.

The great progress of proteomics methodologies allows a deeper investigation of human diseases and their therapies. Today, Proteomics can analyze a wide range of samples, including tissue (fresh, frozen, paraffined), cell lines and biofluids.

Particularly, Proteomics performed with nano liquid chromatography coupled to high-resolution tandem mass spectrometry (nLC-hrMS/MS) represents a powerful tool to explore the molecular mechanisms of pathologies and the cellular alterations induced by diseases, therapies or environmental factors, and to identify potential biomarkers.

Beyond its application for diagnosis, Proteomics has been demonstrated in translational studies as capable of separating responders from non-responders to targeted drugs, such as biologics, and this may enable a more precise approach to therapy, identifying predictive biomarkers associated with treatment response.

In addition, proteomics, such as other omics profiles, can enable identification of altered pathways, and that such pathways are modified by lifestyle.

Molecular and Personalized/Precision Medicine approach is fundamental for understanding resistance mechanisms and identifying related biomarkers to stratify patients, developing appropriate treatment strategies tailored to the subjects and their response to therapy, including radiotherapy.

Also, Systems Biology approaches based on omics technologies are becoming essential tools for the molecular characterization of specific pathophysiological conditions with diagnostic, prognostic and therapeutic purposes.

Specifically, it is important to combine Fundamental Research, for elucidation of molecular mechanisms (endotypes) related to diseases and their therapies, and Translational Research, for discovery of biomarkers useful for diagnosis, phenotyping and therapy monitoring.

Future perspective includes the identification of biomarkers of overexpressed proteins in responders and non-responders, so that they can be easily measured in simple biological samples to collect and to analyze.

In this context, it is of primary importance both the collaboration between hospitals, biobanks and research centers and the availability of *state of art* infrastructures; these accelerate the development of new biomarkers for diagnosis and treatment, with the aim of improving clinical management and more effective therapies.

In the presentation it will be shown some actual translational applications based on Proteomics.

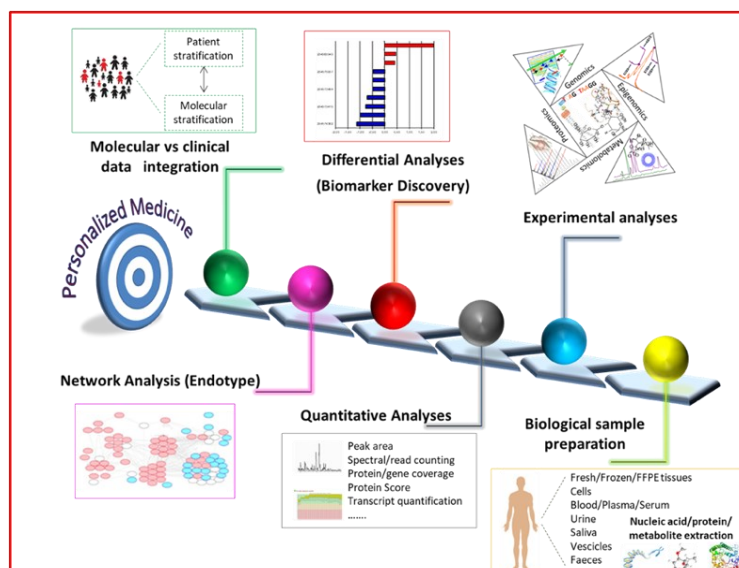


Figure 1. Flow chart combining Fundamental and Translational Activities based on Proteomics

Mass spectrometry trends based on sustainability, environmental impact, and new tools for assessing adherence to green analytical chemistry principles

Marcello Locatelli¹

¹ *Department of Science, University “G. d’Annunzio” of Chieti-Pescara, Chieti, Italy*

Keywords: *Green Profile Tool, Analytical Method Evaluation, White Analytical Chemistry, Mass Spectrometry*

The growing focus on sustainability and safety in analytical laboratories, particularly in light of the principles of Green Analytical Chemistry (GAC), Green Sample Preparation (GSP), and Circular Analytical Chemistry (CAC), has led to the development and adoption of new tools and approaches aligned with these principles throughout the analytical process (from sample to sampling, from sample preparation to instrumental analysis) in order to standardize and harmonize the evaluation of new methods and protocols being developed.

This presentation aims to provide a critical overview of the main tools, accepted by the International Scientific Community, used in analytical chemistry to reduce environmental impact, improve efficiency, and simultaneously ensure high analytical performance and protect operator health. Continuous training and ongoing research have allowed us to increase the number of instruments available, especially in the last two years, focusing not only on green and white analytical chemistry but also on performance and practicality.

This presentation focuses on the latest instruments, analysing their advantages and disadvantages, approaches, innovations, and ease of use, especially related to the MS and LC-MS instrumental platform and the recent trends in terms of methods and configurations.



Figure 1. Comparison between Green Analytical Chemistry (GAC) and White Analytical Chemistry (WAC)

High-Resolution Mass Spectrometry in Supramolecular Chemistry: An Essential Tool for Characterizing Host@Guest Complexes and Self-Assembled Architectures

Paolo Della Sala¹

¹ *Dipartimento di Chimica e Biologia, Università degli Studi di Salerno, Via Giovanni Paolo II 132, 84084 Fisciano, Italy*

Keywords: *Host:Guest Complexes, Self-Assembled Architectures, Molecular Recognition*

Nature harnesses molecular confinement and non-covalent interactions to regulate molecular recognition events and chemical transformations with remarkable efficiency and selectivity [1]. Inspired by these processes, supramolecular chemists have developed various architectures, including capsules, macrocycles, and mechanically interlocked systems, which can mimic natural systems through secondary interactions and nanoconfined environments [2].

A comprehensive investigation of these dynamic assemblies requires analytical techniques capable of probing molecular recognition, stoichiometry, stability, and self-assembly processes with high sensitivity and resolution [3]. In this context, mass spectrometry has emerged as a powerful characterization tool, extending beyond simple mass determination to provide unique insights into solvation phenomena, non-covalent interactions, molecular recognition, and the forces governing the stability of supramolecular assemblies in the gas phase [4].

This contribution highlights the application of high-resolution mass spectrometry, particularly MS-FT-ICR techniques, in studying host-guest complexes and self-assembled supramolecular architectures. Selected examples include self-assembled resorcin[4]arene capsules, host-guest complexes [5], and mechanically interlocked systems, which illustrate mass spectrometry's capacity to reveal detailed insights into encapsulation phenomena, ion-pair interactions, and chiral recognition processes [6].

Overall, the studies presented underscore how combining supramolecular chemistry and mass spectrometry provides significant opportunities for understanding supramolecular systems in both self-assembled capsules and host-guest complexes

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Oral Presentations

Comprehensive UHPLC–HRMS Workflow for Nitrosamine Profiling in Meat Products: Formation Mechanisms and Dietary Implications

Tiziana Nardin¹, Jakob Franceschini¹, Francesca Martinelli¹, Roberto Larcher¹

¹ *Fondazione Edmund Mach, Centro Trasferimento Tecnologico, San Michele all'Adige (TN), Italy*

Keywords: Nitrosamines, UHPLC-HRMS, APCI, HESI, meat products, digestion, food safety

Nitrosamines (NAs) are a class of potentially carcinogenic compounds formed through the nitrosation of secondary amines, particularly in processed meat products where nitrites and nitrates are widely used as preservatives. Despite increasing regulatory attention, their comprehensive detection remains analytically challenging due to their chemical diversity, low concentration levels, and instability.

In this study, an integrated Ultra-High-Performance Liquid Chromatography-High-Resolution Mass Spectrometry (UHPLC–HRMS) workflow was developed for the simultaneous detection of both volatile (VNAs) and non-volatile nitrosamines (NVNAs) in complex meat matrices. The method combines optimized extraction and clean-up procedures with online solid-phase extraction (SPE) and high-resolution Orbitrap detection. A key methodological aspect was the comparison of two ionization sources, heated electrospray ionization (HESI) and atmospheric pressure chemical ionization (APCI), which demonstrated complementary performance.

The method was applied to investigate nitrosamine formation across different conditions, including raw, processed, cooked, and in vitro digested meat samples. Results revealed baseline levels in untreated meat (~3 µg/kg) and significant increases upon nitrite/nitrate addition. Simulated gastric digestion emerged as the most critical factor promoting formation, while cooking alone had limited impact. Ascorbate showed a dual role, acting as an inhibitor at low nitrite levels but promoting formation at higher concentrations.

Statistical analysis confirmed that preservative formulation was the primary driver of variability. NDMA emerged as a key marker of dietary exposure. Overall, this study highlights the analytical complexity and multifactorial nature of nitrosamine formation, providing a robust UHPLC–HRMS approach for comprehensive profiling and risk assessment.

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IR Ion Spectroscopy and Mass Spectrometry reveal surprising Zn(II)-Mediated Naringenin Reactivity

Davide Corinti¹, Lucretia Rotari¹, Valeria Vergine¹, Giel Berden², Jos Oomens², Barbara Chiavarino¹, Maria Elisa Crestoni¹

¹ *Dipartimento di Chimica e Tecnologie del Farmaco, Sapienza Università di Roma, Roma, 00185, Italia*

² *HFML-FELIX, Nijmegen, 6525ED, Netherlands*

Keywords: *coordination chemistry, structure elucidation, antioxidants, isomerization*

Flavonoids are ubiquitous plant polyphenols whose biological activity is closely tied to their ability to quench radicals and chelate metal ions. Among them, naringenin (Nar) is a key flavanone intermediate in biosynthetic pathways and a widely studied nutraceutical molecule with multiple health-related properties. Understanding its metal-binding preferences and the consequences on reactivity of complexation is essential for elucidating its antioxidant and pharmacological functions.

Here we report a detailed gas-phase characterization of 1:1 complexes formed between Zn(II) and the conjugate bases of naringenin and its structural isomer chalcone, $[\text{Zn}(\text{Nar-H})]^+$ and $[\text{Zn}(\text{ChNar-H})]^+$, respectively [1]. The ions were generated by electrospray ionization and structurally interrogated through infrared multiple photon dissociation (IRMPD) spectroscopy, supported by DFT calculations. IRMPD spectra provided diagnostic signatures of the coordination sites while unveiling that Zn(II) catalyzes the interconversion between Nar and ChNar, in fact both $[\text{Zn}(\text{Nar-H})]^+$ and $[\text{Zn}(\text{ChNar-H})]^+$ show identical IR features [2].

An interesting outcome of this study is the oxidative transformation of naringenin upon activation of its Zn(II) complex. CID and wavelength-selective IRMPD both produce fragment ions whose IR signatures matched a combination of protonated genistein and apigenin, revealing that naringenin undergoes gas-phase dehydrogenation and aromatization under mild activation when coordinated to Zn(II). This reactivity, although previously known as a possible metal-promoted oxidative pathway, was believed to yield only protonated apigenin. Our evidence instead points to the additional formation of the structural isomer genistein, produced through a 1,2-phenol shift. Potential energy surface calculations for the dissociation pathways were carried out to rationalize the experimental observations.

Overall, our combined MS/IRMPD/DFT approach elucidates metal–ligand binding motifs, clarifies coordination behavior, and uncovers a remarkable metal-mediated oxidation process with implications for flavonoid reactivity and biological function.

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Disclosing the role of wholemeal semolina and drying conditions on the protein profile and digestibility of durum wheat pasta by HR-MS/MS

Antonella Lamonaca¹, Chiara Nitride², Elisabetta De Angelis¹, Rosa Pilolli¹

¹ Institute of Sciences of Food Production, National Research Council, Via Amendola 122/O, 70126 Bari, Italy

² Department of Agricultural Sciences, University of Naples Federico II, Via Università 100, 80055 Portici, Italy

Keywords: pasta drying cycles, Maillard Adducts, proteomic profile, mass spectrometry

Pasta is a widely consumed food, traditionally appreciated for its complex carbohydrate content. However, aspects related to protein digestion, although important for the nutritional value and functional properties of the product, have been studied much less than starch digestibility. Current studies do not fully describe the organization of wheat proteins or how processing affects them [1]. Thermal conditions applied for pasta drying can trigger Maillard reactions capable of modifying the protein fraction and influencing the sensory and nutritional properties of pasta [2]. The use of wholemeal semolina adds further complexity, as the release of functional molecules during digestion can influence protein degradation [3]. In this context, our study aimed to understand how raw material characteristics and drying conditions influence the protein profile of wholegrain pasta, their thermal modifications (oxidation, deamidation, and changes related to the Maillard reaction), and their fate during human gastroduodenal (GD) digestion simulated *in vitro*. For this purpose, two wholemeal semolina samples (WS A and WS B) with peculiar differences in proximate analysis were used to produce wholegrain spaghetti processed with two drying cycles (21h at 50°C and 6h at 85°C). Proteins were extracted and analysed using a typical shotgun proteomics approach with discovery HR-MS/MS analysis. Peptide identification was performed using an optimized bioinformatics workflow to compare the protein profiles of the samples and detect the Maillard adducts on lysine and other chemical modifications on labile amino acids. Cooked spaghetti samples were then subjected to static *in vitro* GD digestion according to the INFOGEST protocol [4] and investigated using proteomic analysis. Analysis of WS A and WS B allowed us to define their protein composition. Although the semolina samples displayed a largely comparable protein profile, they differed in approximately 15% of the identified proteins, belonging to different functional categories such as enzymatic, structural, storage and transport proteins. In the wholegrain pasta samples, total protein content assayed on dry weight remained similar to the corresponding semolina, but proteomic analysis revealed a marked reduction in identified peptides, with a stronger decrease at higher drying temperatures. This likely reflected reduced protein extractability in the dry pasta. A slight increase in the modified peptides was also observed, particularly those affected by thermal reactions, indicating that stronger treatments promote such modifications. Differential analysis showed that native peptides were the main contributors to significant differences between spaghetti dried at 50°C and 85°C. For digested wholegrain pasta, the degree of protein hydrolysis did not show significant differences between the samples, while digesta profiles showed a predominance of native peptides. Multivariate analysis highlighted differences between raw and digested samples, due to cooking effects and to the variable susceptibility of different protein classes to enzymatic degradation. The study clarified the main role of the wholemeal semolina to the overall characteristics of the derived pasta with a minimal difference in the impact of the two drying temperatures on the final commodities.

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Comprehensive Phenolic Profiling of *Reynoutria* × *bohemica* via LC-ESI-MS: Valorizing Invasive Biomass

Mathieu Merlet¹, Tania Flutto¹, Simona Leveque¹, Sabina Valentini¹

¹ *Institut Agricole Régional, 11100 Aosta, Italy*

Keywords: *Reynoutria* spp., LC-ESI-MS, circular economy, biodiversity, bioactive compounds

Reynoutria spp. are invasive weeds threatening biodiversity due to their adaptability to various soil types and environments. They reduce species richness through allelopathy and possess high dispersal potential. Furthermore, hybridization produces genetically variable and even more invasive species [1]. In the Aosta Valley, the most prevalent taxon is the hybrid *Reynoutria* × *bohemica* (*R. sachalinensis* × *R. japonica*), known for its significant invasiveness. Despite its ecological impact, this plant is a rich source of flavonoids, stilbenes, anthraquinones, and coumarins [2], which exhibit antimicrobial, antiviral, and anti-inflammatory activities [3]. This work aims to characterize the phenolic profile of leaves, stems, and rhizomes of *R. × bohemica* to support management strategies that convert invasive biomass into high-value products for food, cosmetic, or nutraceutical applications.

R. × bohemica samples were collected in June (Nus, Aosta Valley), dried, and milled. Polyphenols were extracted via Accelerated Solvent Extraction (ASE) [4] using varying water/ethanol ratios. Extracts were screened for Total Polyphenolic Content (TPC) and Antioxidant Activity (AOA) via spectrophotometric assays. Detailed phenolic profiling was performed by LC-ESI-MS, with data processing via MS-DIAL software and metabolite identification supported by external standards.

Results indicate high TPC and AOA, particularly in leaf and rhizome extracts (TPC: 12543.2 and 9898.52 mg/L; AOA: 93.84 and 53.64 mg TE/g, respectively) using 80% ethanol and 3 static cycles at 90°C. PCA analysis clearly discriminated the rhizome from the leaf/stem profiles based on their chemical markers: the rhizome was characterized by high concentrations of stilbenes and anthraquinones (e.g., piceid 1443.36 mg/L, resveratrol 580.03 mg/L, emodin 101.24 mg/L), while leaves were enriched in flavonols (e.g., kaempferol 3-glucoside 362.56 mg/L, quercetin 3-glucoside 120.45 mg/L). High levels of catechin, epicatechin, and procyanidin B2 were ubiquitous across all tissues.

Our findings demonstrate that *R. × bohemica* biomass from the Aosta Valley represents a potent, low-cost source of bioactive compounds. The distinct chemical compartmentalization observed suggests tailored valorisation strategies: rhizomes for high quantity of stilbenes (nutraceuticals) and leaves for antioxidant-rich flavonol fractions (cosmetics/plant protection). Future studies will focus on testing the in vitro efficacy of these extracts against specific agricultural pathogens to develop local, bio-based pesticides, thereby integrating invasive species management with the principles of a circular bioeconomy.

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Chemical characterization and biological activity of *Myrtus communis* L. leaves: development of a NADES-UAE extract

Stefania Pagliari¹, Alice Piacentini¹, Matteo Emilio Federico Capietti¹, Hajar Lamkthanter¹, Massimo Labra¹, Luca Campone¹

¹ Department of Biotechnology and Biosciences, University of Milano-Bicocca, Piazza della Scienza 2, 20126 Milano, Italy

Keywords: NADES, *Myrtus communis*, LC-MS, polyphenols, antioxidant

Myrtle (*Myrtus communis* L.) is an aromatic Mediterranean plant that is rich in polyphenols and has well-known therapeutic properties [1]. However, conventional extraction methods for recovering metabolites present many limitations such as long processing times, low extraction yields, and the use of toxic solvents. This study proposes a sustainable method of recovering bioactive compounds from myrtle leaves using Natural Deep Eutectic Solvents (NADES). These solvents were combined with ultrasound-assisted extraction (UAE), to improve extraction efficiency.

First, the efficiency of five different NADES mixtures in recovering bioactive compounds was evaluated using spectrophotometric assays to evaluate antioxidant activity (DPPH, ABTS), total phenolic content and hypoglycaemic effect (α -glucosidase inhibition). Once the most promising combination had been identified, the critical process parameters (temperature, number of cycles, HBA/HBD molar ratio and percentage of water) were optimized using a multivariate experimental design (RSM) to maximise bioactivity. The extract obtained under optimal conditions was then compared with UAE extraction using ethanol, a commonly used solvent for recovering phenolic compounds, and traditional extraction (maceration) in methanol, confirming the greater efficacy of NADES in recovering polyphenols.

The correlation between bioactivity and the chemical characterization using HPLC-ESI-Orbitrap-MS/MS allowed us to identify the main metabolites responsible for the biological activity, especially those belonging to catechin, catechin derivatives, and phenolic glycosides. Finally, analysis of the greenness index and the energy impact showed that the NADES-UAE model guarantees higher yields and represents a significantly greener, more sustainable option for the recovery of polyphenols as ingredient for nutraceutical industry.

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Isotopic application of the High-Resolution Orbitrap Mass Spectrometry: a case study on curcumin

Silvia Pianezze¹, Domenico Masuero², Roberto Penasa², Matteo Perini¹, Alberto Roncone², Luana Bontempo²

¹ Technology Transfer Center, Fondazione Edmund Mach, San Michele all'Adige, Italy

² Research and Innovation Center, Fondazione Edmund Mach, San Michele all'Adige, Italy

Keywords: High-Resolution Orbitrap Mass Spectrometry, Curcumin, carbon isotopic ratio, clumped isotopes

In the present work, the High-Resolution Orbitrap Mass Spectrometry has been applied for the isotopic measurement of curcumin in curcuminoids samples of natural (7), synthetic (6) and unknown (4) origin. Together with the most investigated carbon isotopic ratio ($\delta^{13}\text{C}$), clumped isotopes $^{13}\text{C}^{13}\text{C}$ were also considered, giving first reference values for this parameter ($\delta^{13}\text{C}^{13}\text{C}$). The isotopic abundances of were measured both in curcumin (through an M0 experiment, Figure 1) and in four major curcumin fragments (through an MS² experiment).

Mean $\delta^{13}\text{C}_{\text{VPDB, Orbi}}$ values of $-31.3 \pm 1.0\text{‰}$ and $-27.9 \pm 1.9\text{‰}$ were obtained for natural and synthetic samples, respectively. As for the clumped isotopes, whose isotopic values are calculated against the standard selected for the present study, mean $\delta^{13}\text{C}^{13}\text{C}_{\text{STND, Orbi}}$ of $-3.2 \pm 3.5\text{‰}$ and $+3.2 \pm 4.6\text{‰}$ were obtained for natural and synthetic samples, respectively. The $\delta^{13}\text{C}_{\text{VPDB, Orbi}}$ and the $\delta^{13}\text{C}^{13}\text{C}_{\text{STND, Orbi}}$ of curcumin were linearly correlated (Pearsons' coefficient = 0.93) and they were effective in the discrimination between natural and synthetic samples ($p < 0.05$).

Moreover, fragmentation experiments gave the opportunity to further investigate the isotopic distribution along curcumin structure, while the $\delta^{13}\text{C}_{\text{STND, Orbi}}$ and $\delta^{13}\text{C}^{13}\text{C}_{\text{STND, Orbi}}$ of most fragments were statistically different between natural and synthetic samples ($p < 0.05$). The combination of the results obtained through the M0 and the MS² experiments provided a promising tool for curcumin authentication.

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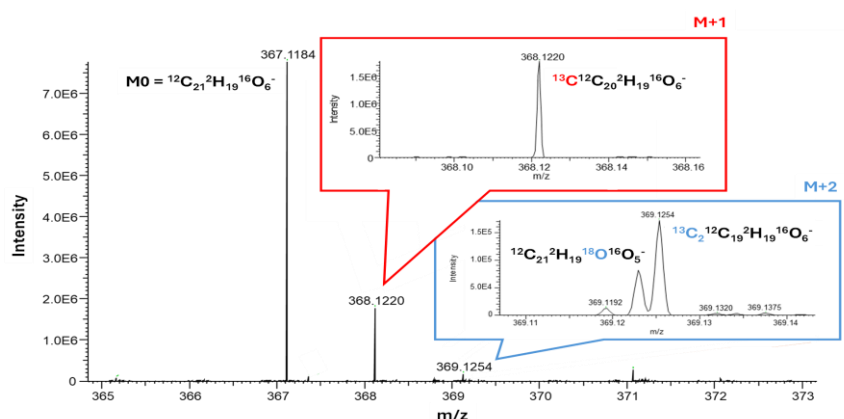


Figure 1. Mass spectrum of the M0 experiment: 367.1184 m/z = molecular peak M0; 368.1220 m/z = $^{13}\text{C}^{12}\text{C}_{20}\text{H}_{19}\text{O}_6^-$; 369.1254 m/z = $^{13}\text{C}_2^{12}\text{C}_{19}\text{H}_{19}\text{O}_6^-$.

An Optimized IM-MS Workflow for the Analysis of Native N-Glycans from Human Plasma

Claudia Fumagalli¹, Lisa Pagani¹, Clizia Chinello¹, Nicole Monza¹, Felisia Di Nicoli¹, Fulvio Magni¹, Vanna Denti¹

¹ *Proteomics and Metabolomics Unit, Department of Medicine and Surgery, University of Milano-Bicocca*

Keywords: *Glycomics, Nano-flow liquid chromatography, Ion mobility-mass spectrometry*

Glycomics has gained increasing attention in recent years, as glycosylation represents one of the most abundant and structurally complex forms of post-translational modification. N-glycans play a crucial role in regulating protein function, immune surveillance, inflammatory responses, and hormonal activity [1]. Alterations in glycosylation patterns have been strongly associated with a wide range of diseases, including cancer, autoimmune disorders, and neurodegenerative and cardiovascular conditions, often preceding the onset of clinical symptoms, highlighting their potential as early biomarkers. However, comprehensive N-glycome analysis remains challenging due to the intrinsic limitations of mass spectrometry (MS) alone, particularly in resolving isomeric structures that share identical mass-to-charge ratios (m/z). Recent advances in ion mobility (IM) coupled with MS (IM-MS), particularly with trapped ion mobility spectrometry (TIMS) have introduced an additional dimension of separation based on molecular shape and collision cross section (CCS), significantly enhancing the discrimination of glycan isomers and improving confidence in structural annotation [2]. In this work, we focus on the optimization of an IM-MS workflow using a timsTOF flex instrument for the characterization of complex N-glycans released from plasma proteins in their native state, with the final aim of building a comprehensive database that will be used for clinical investigations. Sample preparation was first optimized by evaluating different purification strategies, including variations in incubation time and temperature during PNGase F digestion, as well as solid-phase extraction protocols. In parallel, a nano-liquid chromatography (n-LC) separation method was optimized on a set of 12 released N-glycans standards. Despite the most widely adopted approaches for N-glycan separation typically involve hydrophilic interaction liquid chromatography (HILIC) columns [1], it did not provide an adequate separation of the analytes with our instrumental setup. Therefore, an alternative method based on a reverse phase (RP) PepSep C18 15cm x 75 μ m, 1.9 μ m nanocolumn, was developed and optimized. Simultaneously, IM-MS optimization was required, since no comparable methods were available in literature, particularly as conventional glycomics workflows often include derivatization steps, which were intentionally omitted in our study. To do so, extensive optimization of TIMS parameters, using the 12 standards as reference, including the optimal mobility window, duty cycle, accumulation time and collision energy was performed. As a result, the optimized workflow enabled the separation of isomeric N-glycans without the need for derivatization and allowed the acquisition of highly informative MS/MS spectra, leading to the generation of a first plasma glycome dataset. Data analysis was lastly performed using PEAKS GlycanFinder, a powerful tool for database-assisted and de novo glycan identification. This dataset can also support complementary MS experiments, which provide spatially resolved but often structurally ambiguous information. Ultimately, this workflow aims to create a database with 4 variables for each N-glycan, including retention time, m/z , MS/MS spectrum and CCS value and apply the whole workflow to dried blood spot samples for biomarker discovery.

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Uncovering Early Renal Alterations in Fabry Disease through Spatial Proteomics

Natalia Shelly Porto¹, Greta Bindi¹, Vincenzo L'Imperio², Fabio Pagni², Fulvio Magni¹, Andrew Smith¹

¹ *Proteomics and Metabolomics Unit, School of Medicine and Surgery, University of Milano Bicocca, 20854 Veduggio al Lambro, Italy*

² *Department of Medicine and Surgery, Pathology, IRCCS Fondazione San Gerardo dei Tintori, University of Milano-Bicocca, Monza 20900 MB, Italy*

Keywords: Zebrafish, MALDI-MSI, Fabry disease

Fabry disease (FD) is the second most common inherited lysosomal storage disorder after Gaucher disease and is caused by mutations in the *GLA* gene on the X chromosome, leading to reduced or absent activity of the enzyme α -galactosidase A (α -GAL A). This results in the progressive accumulation of globotriaosylceramide (Gb3) in lysosomes [1]. FD incidence varies by sex, with males more severely affected due to X-linked inheritance [2]. Classically affected hemizygous males, who lack residual α -GAL A activity, may exhibit hallmark features of the disease, including neurological, cutaneous, renal, cardiovascular, cochleo-vestibular, and cerebrovascular manifestations [3]. Although FD presents with multisystemic involvement, the kidney is one of the most severely affected organs. Gb3 accumulation leads to progressive renal dysfunction, initially characterized by hyperfiltration and potentially evolving into end-stage renal disease (ESRD) [4]. Given the clinical burden of renal involvement, understanding early molecular events in Fabry nephropathy is crucial for the development of effective therapeutic strategies. Despite advances in treatment, further research is needed to better elucidate the molecular mechanisms underlying FD, highlighting the need for reliable experimental models. In this context, zebrafish (ZF) has emerged as a valuable *in vivo* model for lysosomal storage disorders, as it recapitulates key features of FD pathophysiology, including organ-specific damage, more effectively than other animal models that often lack clear pathological phenotypes [5].

To investigate early molecular alterations associated with renal involvement in FD, we applied an integrated mass spectrometry (MS)-based proteomics approach in a zebrafish model. Specifically, Matrix-Assisted Laser Desorption/Ionization mass spectrometry imaging (MALDI-MSI) was used to achieve spatially resolved proteomic profiling of kidney tissue, preserving tissue architecture while identifying disease-related molecular signatures. This approach was complemented by liquid chromatography tandem mass spectrometry (nLC-ESI-MS/MS), enabling protein identification. This combined strategy allowed the detection of proteomic alterations associated with renal manifestations of FD, identifying discriminative protein signatures related to inflammatory and stress-response pathways, including Caspase a (CASP_A_DANRE), Keratin, type II cytoskeletal 8 (K2C8_DANRE), and Synaptobrevin homolog YKT6 (YKT6_DANRE), even in the absence of overt histological changes. Therefore, this approach provides a powerful tool to uncover early molecular indicators of renal damage and identify proteomic signatures with potential relevance for disease monitoring.

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A multimatrix LC-MS/MS approach reveals airway exposure-response relationships for elexacaftor/tezacaftor/ivacaftor in cystic fibrosis

Matteo Mucci¹, Pietro Ripani², Marta Di Nicola¹, Martina Colarelli¹, Antonio Recchiuti¹

¹ Department of Medical, Oral, and Biotechnology Science (DSMOB), University "G. d'Annunzio" of Chieti-Pescara, Center of Advanced Studies and Technology, 66100 Chieti, Italy

² Regional CF Center, San Liberatore Hospital - ASL Teramo, Atri, Italy, Italy

Keywords: cystic fibrosis; LC-MS/MS; therapeutic drug monitoring; nasal airway swab; dried blood spot; CFTR modulators

Clinical response to fixed-dose elexacaftor/tezacaftor/ivacaftor (ETI) remains markedly heterogeneous among people with cystic fibrosis (CF), and this variability is only partly explained by baseline clinical characteristics [1]. Standard plasma-based therapeutic drug monitoring is analytically feasible but may not adequately reflect drug exposure at the airway epithelium, one of the main pharmacological targets of CFTR modulators. To address this limitation, we developed and validated, according to ICH M10 guidelines, a multimatrix LC-MS/MS method for ETI quantification in conventional plasma as well as in minimally invasive volumetric dried blood spots (DBS) and nasal airway swabs (NAS) [2]. The method showed satisfactory analytical performance across matrices and supported the extension of ETI monitoring from systemic circulation to the airway compartment, providing direct access to the pharmacologically relevant target tissue. Moreover, we applied this platform in a cross-sectional observational study including 40 clinically stable adults with CF receiving standard ETI therapy. DBS and NAS were collected at the same visit, and ETI concentrations were quantified by LC-MS/MS [2]. Associations between ETI concentrations and percent predicted FEV1 (ppFEV1) were evaluated. Clinical response to ETI was heterogeneous: 11/40 participants (27.5%) showed a ppFEV1 gain ≤ 5 points [3.0 (-1.0 to 4.0)], whereas 29/40 achieved 15.0 (11.2-20.5) points ($p < 0.001$). NAS concentrations were about 10-fold higher than systemic measurements. Unlike plasma, NAS concentrations correlated with lung function, with positive associations for elexacaftor ($\rho = 0.33$, $p = 0.035$) and tezacaftor ($\rho = 0.42$, $p = 0.007$), and a similar trend for ivacaftor ($\rho = 0.31$, $p = 0.053$). In adjusted logistic regression, nasal tezacaftor independently classified participants achieving ppFEV1 improvement > 5 points (OR 1.219 per $\mu\text{g/mL}$, 95% CI 1.021-1.450; $p = 0.029$). Overall, this multimatrix LC-MS/MS strategy shows that airway ETI exposure, rather than systemic exposure, better tracks lung function response in adults with CF. NAS sampling is minimally invasive, analytically robust, and directly informative on the target compartment, supporting its use as a translational biomarker for therapeutic drug monitoring, identification of low responders, and future precision dosing of CFTR modulators.

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Single-Step DIA-PASEF Unlocks Comprehensive Antigen Profiling in Membranous Nephropathy from Minimal Microdissected Glomeruli

Viola Tintori¹, Clizia Chinello¹, Jan Ulrich Becker², Federico Alberici³, Martina Tedesco³, Vincenzo L'imperio⁴, Andrew Smith¹

¹ *University of Milano-Bicocca, Department of Medicine and Surgery, Proteomics and Metabolomics Unit, Veduggio al Lambro, Italy*

² *Medical University of Vienna, Department of Pathology, Vienna, Austria, 7 University Hospital of Cologne, Institute of Pathology, Cologne, Germany*

³ *ASST Spedali Civili di Brescia, Nephrology Unit, Spedali Civili Hospital, Brescia, Italy*

⁴ *Fondazione IRCCS San Gerardo dei Tintori, Nephrology Unit, Monza, Italy*

Keywords: *Membranous nephropathy, Antigen heterogeneity, Mass spectrometry-based proteomics, Quantitative proteomics, Clinical stratification*

Membranous nephropathy (MN) is a rare glomerular disease driven by subepithelial immune complex deposition, leading to podocyte injury and nephrotic syndrome [1]. Current diagnostic practice relies on immunohistochemistry, which is restricted to a limited number of validated antigens, mainly Secretory phospholipase A2 receptor (PLA2R1) and Thrombospondin Type-1 Domain-Containing 7A (THSD7A), leaving a substantial fraction of patients without a definitive diagnosis [2]. This limitation stems from the marked antigenic heterogeneity of MN, with an expanding and incompletely characterized repertoire of pathogenic targets beyond PLA2R1 and THSD7A [3-4]. Despite this, routine diagnostics remain confined to these canonical markers due to the dependence on antibody-based assays, which require additional tissue sections and well-validated reagents that are often unavailable or inconsistently standardized across centres [5]. In combination with limited biopsy material, this prevents comprehensive antigen profiling in routine practice, resulting in a persistent pool of antigen-negative patients and a critical barrier to mechanism-based diagnosis and therapy selection.

Here, we present a clinically compatible, high-sensitivity proteomics workflow that overcomes these limitations by enabling comprehensive antigen profiling in a single experiment. The method integrates laser capture microdissection (LCM) [6] with nano liquid chromatography–tandem mass spectrometry (nanoLC–MS/MS) operating in data-independent acquisition with ion mobility (DIA-PASEF), allowing simultaneous identification and relative quantification of glomerular proteins directly from renal biopsies [7-8]. Method optimisation assessed analytical sensitivity using increasing numbers of microdissected glomeruli (1–45) from FFPE renal biopsies, analysed by DIA-PASEF. Robust proteomic profiles and confident antigen detection were achieved with as few as 3 glomeruli, demonstrating suitability for low-input clinical samples. The optimised workflow was subsequently applied to a multicentre cohort of 23 MN patients, analysing 10 glomeruli per case, a number fully compatible with routine diagnostic workflows. Applied to this cohort, the method showed high reproducibility and achieved 100% concordance with routine clinical classification, correctly identifying the known pathogenic antigen in all cases, thus validating its reliability as a diagnostic-support tool. Crucially, the intrinsic multiplexing capability provides a decisive advantage over antibody-based methods. Each analysis enables the quantification of thousands of proteins, including both established and candidate MN antigens, supporting direct intra-sample comparison and systematic antigen discovery. This approach substantially improves diagnostic yield: in the unresolved subgroup, a candidate antigen was identified in 65% of double-negative patients (11/17), markedly reducing the proportion of unclassified cases. Importantly, the same dataset enables downstream exploratory statistical analyses without additional sample consumption. As proof of concept, comparative analysis between rituximab responders (CR) and non-responders (NR) identified ~70 differentially expressed proteins (30 down-

and 40 up-regulated in NR vs CR), with associated pathway-level differences, demonstrating the feasibility of extracting clinically relevant molecular stratification directly from a single acquisition. Overall, this workflow delivers full diagnostic concordance, minimal sample requirements, and a major increase in antigen identification rate, while simultaneously enabling proteome-wide exploratory analyses. This integrated approach addresses a key unmet clinical need in MN and establishes a scalable platform for precision nephrology, biomarker discovery, and therapy-oriented patient stratification.

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The role of in-source fragmentation patterns in the RPLC–HRMS-based profiling of oxysterols in pancreatic tumour cell models

Vito Nettis¹, Andrea Castellaneta¹, Noa Ndimurwanko^{2,3}, Liam A. McDonnell², Tommaso R.I. Cataldi¹, Cosima D. Calvano¹

¹ Dipartimento di Chimica, Università degli Studi di Bari Aldo Moro, 70126 Bari, Italy

² Fondazione Pisana per la Scienza ONLUS, 56017 San Giuliano Terme, Italy. ³ Scuola Normale Superiore, 56126 Pisa, Italy

Keywords: *Oxysterols, pancreatic cancer cells, extracellular vesicles, high-resolution mass spectrometry, in-source fragmentation*

Pancreatic cancer (PC) has the worst prognosis of all cancers. It remains asymptomatic in the initial stages, which makes it challenging to diagnose early using established screening methods. Pancreatic ductal adenocarcinoma (PDAC) accounts for around 90% of PC cases and is usually diagnosed at an advanced stage, resulting in a five-year survival rate of just 7% [1]. This study focuses on oxysterol's (OS) profiling in healthy and pancreatic tumour cell lines. OS are emerging as active drivers of cancer, shaping aggressive, immunosuppressive tumour niches rather than acting as passive by-products of cholesterol [2]. Here, we developed and applied an advanced liquid chromatography coupled with mass spectrometry (LC–MS) strategy for the comprehensive qualitative and quantitative profiling of the total (free and esterified) OS in healthy pancreatic epithelial (HPNE) cells and pancreatic ductal adenocarcinoma (PANC-1) cells. Both cell types were cultured under normoxic and hypoxic conditions to elucidate the impact of oxygen availability on OS metabolism. OS separation was achieved using two complementary stationary phases, i.e., cyanopropyl (ES-CN) and pentafluorophenyl (F5), providing orthogonal chromatographic selectivity. OS identification relied on retention matching with analytical standards across both columns and was further confirmed by the evaluation of in-source fragmentation patterns. In some cases, the latter were exploited to differentiate isomeric species based on diagnostic signal intensities reflecting the differential thermodynamic stability of product ions. This constitutes an unprecedented and efficient approach to discriminate isomeric species along the MS1 dimension. Overall, analyte assignment was considered reliable only when both retention time matching and concordance of in-source fragmentation patterns with those of the corresponding analytical standards were met. These combined criteria ensured robust distinction between isomeric species and minimised the risk of misidentification. This multidimensional analytical framework ensured the unambiguous screening of up to 14 OS in cells and their extracellular vesicles (EVs). The tumour cell phenotype, together with hypoxic conditions, was associated with a marked accumulation of ring-oxidized OS, including 3 β ,5 α ,6 β -cholestantriol, 7-ketocholesterol, and 7-hydroxycholesterol (HC) epimers, suggesting either a metabolic rewiring or an increased intracellular oxidative environment. In contrast, side-chain OS showed distinct dependencies on cell type and oxygen availability: 24(S)-HC was reduced in tumour cells while the amount of 26(R/S)-HC was increased when both cell types were grown in normoxic conditions. EVs exhibited a unique OS signature dominated by 25-HC and 5 β ,6 β -epoxycholesterol.

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Development of a green LC-MS/MS method for the detection of peanuts allergens in oil samples

Anna Luparelli¹, Laura Quintieri¹, Elena C.L. Rigante¹, Alexandre Verdu², Linda Monaci¹

¹ *Institute of Biomembranes, Bioenergetics and Molecular Biotechnologies, National Research Council (IBIOM-CNR), Bari, Italy*

² *Bruker France S.A.S. Applied Mass Spectrometry, 34 Rue de l'Industrie, 67166 Wissembourg Cedex, France*

Keywords: *Peanut allergens, Oily food matrices, LC-MS/MS MRM analysis*

Cross-contamination of foods by allergens represents a safety risk for allergic consumers [1]. Currently available and routinely used methods are mainly based on immuno assay kits for the detection of allergenic ingredients in food products. On the other hand, application to selected and highly processed foods can raise issues making the whole analysis complex and challenging; this is the case of oil rich products where there is a lack of reliable and sensitive methods available [2,3].

In addition, processing modifies protein structure, and this complicates the identification and quantification of allergens especially in ultra-processed foods. Methods capable of detecting low amounts of allergens in oily matrices, would be needed to answer the question about the amount of residual allergenic proteins in oil, in order to properly estimate the risk for allergic consumers.

In this note we present the optimization of reliable and robust reference LC-MS/MS based methods that can be widely used for the quantification of peanut allergens in oily matrices. We herein describe the optimization of a whole analytical protocol for peanut identification and quantification in oil samples by a stepwise approach. First, sample preparation for the extraction of allergenic proteins from complex lipid matrices was optimized, with a specific focus on edible oils. Two extraction strategies were compared: conventional saline buffers and Deep Eutectic Solvents. Further, MSMS method was duly tuned in order to develop a sensitive targeted MSMS method on a Triple Quadrupole Mass Spectrometer. Method optimization was carried out on sunflower oil spiked with peanut proteins at 1000 ppm. Five saline extraction buffers were compared, and the best extractive conditions were further investigated by MSMS analysis. The overall optimized LC MS method used a EVOQ® DART TQ+ system to enable the simultaneous detection of 27 peanut derived marker peptides. Based on precursor ion acquisition results, a targeted MRM method was built up for the simultaneous quantification of 13 peanut reliable markers, where both the quantifier and the qualifier fragment ions were monitored. The MRM method allowed a sensitive and robust preliminary assessment of the extraction efficiency of the tested protocols.

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Zebrafish model for Sucrosomial® Iron studies

Virginia De Vito¹, Tommaso Angelini¹, Tommaso Tozzini¹, Rosario Licitra², Baldassare Forti², Germano Tarantino¹, Elisa Brilli¹

¹ PharmaNutra S.p.A., Pisa, Italy

² Department of Veterinary Sciences, University of Pisa, Pisa, Italy

Keywords: Isotope ⁵⁷Fe, Sucrosomial® Iron Absorption, *in vivo* Zebrafish Studies

Sucrosomial® Iron is a patented oral iron formulation based on a preparation of ferric pyrophosphate, covered by phospholipids plus a sucrose matrix, which is absorbed through para-cellular and trans-cellular routes (included M cells) [2]. This technology protects iron to the acidic environment of the stomach, preserves Fe³⁺ from reduction by intestinal enzymes, and promotes Fe³⁺ absorption and transport across the intestinal epithelium unmediated by the divalent metal transport 1 (DMT-1) carrier [3]. Zebrafish (*Danio Rerio*) is a vertebrate species with genes and metabolic systems that are highly conserved (80%) with humans, including molecular mechanisms regulating cellular iron transport and metabolism [4]. The aim of this study was to investigate Sucrosomial® Iron absorption using an *in vivo* Zebrafish model. In the first study, the iron absorption was evaluated during the development from wild type embryos to larvae. Iron sulphate (FeSO₄), gold standard, and Sucrosomial® Iron, were studied. Iron treatments were added into the water to embryos from 24 to 120 hpf (hours post fertilization). Hatching rate at 48 hpf and 72 hpf, survival rate and body length (mm) at 120 hpf were examined for the toxicological effects. At 120 hpf larvae were pulled for each treatment group to quantify the iron. Both treatments revealed to be safe and larvae treated with Sucrosomial® Iron (3286 ng/mL) showed to be significantly higher compared to Control (1412 ng/mL) and FeSO₄ (1815 ng/mL) groups. The second *in vivo* study was performed in adult wild type Zebrafish following a multiple treatment of 21 days at the dose of 130 mg/kg BW/day of iron. Due to the high level of endogenous and exogenous iron observed in water, food and whole fish, it was necessary to use a natural isotope present in a lower percentage, ⁵⁷Fe (2,2%), compared to the most abundant ⁵⁶Fe (91,7%). An ICP-MS method was developed and validated for quantification of both isotopes in whole fish and organs (gills, brain and intestine). Sucrosomial® Iron and Sucrosomial® Iron with ⁵⁷Fe showed a comparable concentration trend. Moreover, in whole fish and organs, Sucrosomial Iron ⁵⁷Fe concentration showed to be significantly higher than Control treatment already from the first week of treatment to the end of the experiment (21 days). The use of ⁵⁷Fe isotope was useful to appreciate the real Sucrosomial® Iron Absorption Kinetics in adult Zebrafish. These data confirmed that wild type Zebrafish it's a suitable animal model to study iron adsorption with Sucrosomial Technology®. Future studies on mutant anemic Zebrafish were necessary to confirm our preliminary data in wild type animals.

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Untargeted metabolomics via UPLC-HRMS and ASCA-based statistical modeling to characterize metabolic alterations following acute and repeated bupropion exposure

Ilenia Bracaglia^{1,2}, Camilla Montesano¹, Ludovica Chiodo¹, Francesco Bartolini¹, Fabiana Di Rosa³, Adolfo Gregori³, Matteo Marti⁴, Federico Marini¹, Manuel Sergi¹

¹ Department of Chemistry, Sapienza University of Rome, Rome, Italy

² Department of Public Health and Infectious Diseases, Sapienza University of Rome, 00185, Rome, Italy

³ Carabinieri, Department of Scientific Investigation (RIS), Italy

⁴ Department of Translational Medicine, Section of Legal Medicine and LTTA Centre, University of Ferrara, Italy

Keywords: *New Psychoactive Substances (NPS), non-targeted metabolomics, high-resolution mass spectrometry (HRMS), ANOVA–Simultaneous Component Analysis*

Novel Synthetic Opioids (NSOs) currently represent the third most prevalent and hazardous group among New Psychoactive Substances (NPS), following synthetic cathinones and synthetic cannabinoids. Their extreme pharmacological potency compared to traditional opioids, coupled with structural heterogeneity and low concentrations in biological matrices, poses significant analytical challenges for detection [1]. Exposure to these substances causes severe respiratory and neuroadaptive deficits, necessitating advanced forensic strategies and retrospective analyses to identify them. This study proposes an indirect screening approach based on untargeted metabolomics by LC-HRMS to monitor systemic responses to bupropion. Utilizing an in vivo murine model (n = 20, equally divided between males and females), urinary metabolic profile variations were analyzed following acute and repeated administrations. Samples, collected at 0-12 h and 12-24 h intervals, were processed using ANOVA–Simultaneous Component Analysis (ASCA) [2]. This statistical model allowed for the isolation of metabolic fingerprints associated with distinct experimental factors (sex, treatment frequency, and sampling time), providing a detailed characterization of sex-specific alterations, pharmacokinetic profiles, and mechanisms of adaptation and tolerance. The results reveal a profound metabolic divergence linked to sexual dimorphism: while females exhibit a high capacity for recovery supported by efficient metabolic biotransformation, males undergo a systemic bioenergetic collapse, likely due to greater persistence and accumulation of substances. Male vulnerability is characterized by neuro-metabolic dysfunction, involving the shunting of tryptophan toward the kynurenine pathway (promoting a pro-inflammatory environment), inhibition of GABA release, and altered dopaminergic turnover. The systemic imbalance in males is further defined by massive amino acid and nucleotide catabolism, alongside an energetic deficit exacerbated by inefficient β -oxidation; this leads to acyl-carnitine accumulation and a drastic reduction in 3-hydroxybutyrate (BHB), further reflecting compromised intestinal barrier integrity.

In conclusion, this study validates untargeted metabolomics via LC-HRMS as a robust analytical framework for monitoring NSOs. By circumventing the limitations of direct precursor detection, often hindered by trace-level concentrations, this indirect approach based on endogenous urinary profiling ensures an extended detection window and enhanced diagnostic robustness.

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High-Resolution Mass Spectrometry reveals the effects of water stress on olive xylem chemistry and spittlebug feeding

Maria Assunta Crescenzi¹, Zeinab Hamouche^{2,3}, Vincenzo Verrastro³, Enrico de Lillo², Daniele Cornara², Paola Montoro¹

¹ Department of Pharmacy, University of the Study of Salerno, Via Giovanni Paolo II 132, 84084 Fisciano, Italy

² Department of Soil, Plant and Food Science (DiSSPA), University of Bari, Via Amendola 165/A, 70126 Bari, Italy

³ CIHEAM-Bari, Via Ceglie 9, 70010 Valenzano (BA), Italy

Keywords: Xylem Metabolomics, Water Stress, HR-MS/MS

Water stress, increasingly frequent under climate change scenarios, can profoundly influence plant–insect interactions and the transmission dynamics of plant pathogens. In this study, we investigated how plant water status modulates the interaction between the xylem-feeding insect *Philaenus spumarius* and two olive cultivars, Leccino and Ogliarola, which differ in drought tolerance and vector preference. Emphasis was placed on the application of high-resolution mass spectrometry to unravel xylem sap metabolic changes underlying these interactions. Host plant acceptance was assessed by Electrical Penetration Graph (EPG), while the chemical composition of xylem sap was comprehensively profiled by untargeted metabolomics based on UHPLC-HR-MS/MS (Q-Exactive platform) [1]. This mass spectrometry–driven approach enabled high-sensitivity detection and annotation of a broad range of primary and secondary metabolites, providing deep insight into stress-induced metabolic reprogramming [2]. Mass spectrometry data, processed through advanced chemometric workflows (MZmine and multivariate analysis), revealed a clear cultivar-dependent response to water stress. Ogliarola exhibited a remarkably stable xylem metabolome across irrigation regimes, whereas Leccino showed pronounced metabolic plasticity. In Leccino, severe water stress triggered a distinct shift from primary metabolism - characterized by reduced organic acids - to the accumulation of secondary metabolites, including secoiridoid glucosides, lignans, and phenolic compounds, robustly captured and discriminated by HR-MS/MS profiling. These metabolomic changes were associated with altered insect feeding behaviour, with increased xylem ingestion on severely stressed Leccino plants, indicating enhanced host suitability. Notably, mass spectrometry enabled the identification of metabolite classes that potentially modulate feeding, highlighting the central roles of organic acids and specialized metabolites [3]. Overall, this study demonstrates the critical contribution of high-resolution mass spectrometry–based metabolomics in linking plant physiological status to vector behaviour. These findings suggest that water management practices may indirectly influence the spread of *Xylella fastidiosa* by reshaping xylem chemistry, emphasizing the importance of MS-driven approaches in plant-vector interaction studies.

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An Integrated Quantification in Soil from Legacy to Ultra-Short Chain PFAS: A Multi-Platform Mass Spectrometry Strategy Optimized via Design of Experiments

Michela Torrelli^{1,2}, Consolato Schiavone¹, Francesco Romaniello¹, Maria Assunta Acquavia³, Alberto Salomone², Andrea Mario Rossi¹, Chiara Portesi¹

¹ *Istituto Nazionale di Ricerca Metrologica, Strada delle Cacce, 91 10135 Torino, Italy*

² *Department of Chemistry, University of Turin, Via Giuria, 7 10125 Torino, Italy*

³ *Istituto Nazionale di Ricerca Metrologica, Via San Rocco, 1 75100 Matera, Italy*

Keywords: *PFAS, Soil, USC-PFAS, Design of Experiments, Mass Spectrometry*

Per- and polyfluoroalkyl substances (PFAS) are widely used synthetic compounds whose persistence and toxicity pose a growing concern for human health. Due to their distribution within ecosystems, soil has become the main reservoir for PFAS [1]. Interaction between soil and air, water, and plants can be a significant source of PFAS exposure for humans. The sorption and the kind of PFAS that can be absorbed are influenced by elements including pH, organic matter and minerals, and the soil's cation and anion exchange capacities [2]. This work aims to develop, optimize, and validate an analytical method of extraction and analysis of 39 PFAS, including the Ultra-Short Chain (USC) PFAS. Specifically, a Design of Experiments (DoE) strategy, based on a Face-Centered Central Composite Design, was applied to optimize the extraction recoveries of various PFAS classes by varying different extraction parameters, such as salt molarity, pH, and sonication time. The constructed response surface indicated the presence of an optimum region within the experimental domain. Analyses were performed using both High-Resolution Mass Spectrometry to quantify classic PFAS and Triple Quadrupole Mass Spectrometry for USC-PFAS. This ensured comprehensive detection across 60 diverse agricultural soil samples from different geographical areas. In both instruments, optimizing the chromatographic conditions and using a delay column ensured the efficient analyte separation, demonstrating high sensitivity and good linearity. These approaches enabled us to achieve Apparent Recoveries% ranging from 80% to 120% and a fit-for-purpose LOQ of 0.050 µg/kg for most analytes, including PFOA and PFOS, the principal soil contaminants. The results showed PFOA and PFOS contamination in all samples analysed, with concentrations between 0.086 µg/kg and 0.746 µg/kg for PFOA and 0.070 µg/kg and 1.579 µg/kg for PFOS. Furthermore, while the specific PFAS profiles varied, no soil was found to be free of contamination. The final evaluation confirmed that the use of DoE made it possible to extract and quantify a large panel of PFAS with minimal difference.

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Comprehensive GC-MS Profiling of Glandular Secretions in Wild Wolves (*Canis lupus*): Characterization of Volatile and Semi-Volatile Semiochemicals

Serena Fiorito¹, Chiara Colavecchio¹, Francesco Epifano¹, Salvatore Genovese¹

¹ Dipartimento di Farmacia, Università degli Studi "G. d'Annunzio" di Chieti-Pescara, 66100 Chieti, Italy

Keywords: Gas chromatography–mass spectrometry, *Canis lupus*, Gland secretions, Volatile and semi-volatile compounds, Chemical signals, Principal component analysis

Volatile and semi-volatile chemical profiles from glandular secretions of wild wolves (*Canis lupus*) were characterized using an optimized Gas Chromatography–Mass Spectrometry (GC–MS) method combined with Principal Component Analysis (PCA). Biological samples were collected non-invasively from 11 road-killed individuals in the Abruzzo region (Italy), including adult males, adult females, and male juveniles. Samples were taken from the perianal, preputial, interdigital, Harderian, and salivary glands. Samples were extracted with *n*-hexane, derivatized, and analysed by GC–MS to maximize separation efficiency and detection sensitivity for structurally diverse volatile and semi-volatile compounds.

A total of 178 compounds were identified, including hydrocarbons (*n*-alkanes C23–C54), alcohols, aldehydes, carboxylic acids, esters, saturated and unsaturated fatty acids, sterols, and steroid-related metabolites.

Despite significant advances in chromatographic environmental analysis, its application to wildlife monitoring and ecological biomarker discovery remains underdeveloped [1]. Standard workflows are often challenged by complex biological matrices, low analyte concentrations, and the need for tailored sample preparation protocols that preserve both chemical and biological integrity [2].

In this study, gland-specific chemical profiles were clearly differentiated, with perianal and male preputial glands exhibiting the highest compound diversity and abundance. Sex- and age-related differences were evident, particularly in unsaturated fatty acids and steroid derivatives, whereas juvenile samples retained a conserved chemical profile with distinct relative abundances.

Principal component analysis enabled robust discrimination of samples according to gland type, sex, and age class, identifying the key chemical classes driving group separation. This integrated GC–MS/PCA workflow demonstrates reproducible analytical performance for resolving complex biological matrices and provides a robust methodological framework for investigating chemical communication and ecological biomarker discovery in wild carnivore populations.

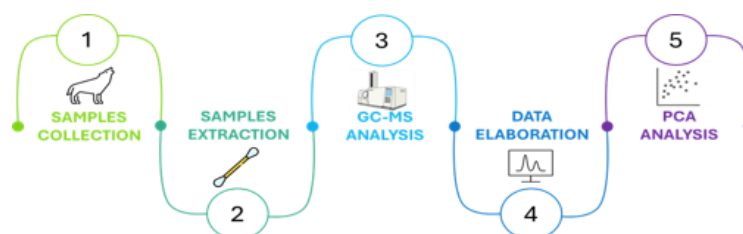


Figure 1. General workflow

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Direct classification of Essential Oils with a Miniature, Deployable Mass Spectrometer Equipped with a Cold Plasma Ion Source

Daniela Peroni¹, Eligio Sebastiani¹, Enrico Davoli^{1,2}

¹ *SRA Instruments SPA, Via alla Castellana 3, 20063 Cernusco sul Naviglio, Milano, Italy*

² *Dipartimento di Biotecnologie Molecolari e Scienze per la Salute, Università di Torino, 10126 Torino, Italy*

In recent years, significant progress has enabled the miniaturization of mass spectrometry instruments.. Small instruments are now commercially available for analytical applications in various fields. However, to achieve portability, some mass spectrometry (MS) performance characteristics—such as resolution, sensitivity, or the ability to operate in MS/MS mode—are often compromised. In this work we present an innovative field-deployable MS (MTE30, MassTech Inc., Columbia) equipped with an atmospheric-pressure interface, coupled with a ring-shaped cold plasma ion source (SICRIT, Plasmion, Augsburg) and a Solid-Phase Microextraction (SPME) sampler. The instrument is fully integrated into a single chassis, requires no external pumps or gases, weighs approximately 17 kg, and provides better-than-unit mass resolution over a 2000 Da range. MS/MS acquisitions are also possible, enhancing instrumental specificity for complex samples.

The SICRIT ion source (Soft Ionization by Chemical Reaction In Transfer) is a general-purpose ion source that can also be installed on conventional laboratory-grade LC/MS instruments to perform direct analysis or GC analysis of VOCs when higher sensitivity or specificity is required. The SPME capability also enables trace and ultra-trace odorant VOC analysis in air samples

This study demonstrates the direct classification of essential oils with the MTE30 MS using the SICRIT cold plasma ion source. Eleven essential oil samples - five lavender oils, five rosemary oils, and one chamomile oil - were analyzed in approximately five minutes, revealing differences in both composition and relative signal intensities. Classification of the oils can be achieved through chemometric approaches following appropriate model training.

Determination of pendimethalin metabolites in zebrafish using *in silico* prediction coupled with high-resolution mass spectrometry

**Fabiola Eugelio¹, Marinella Tano¹, Sara Palmieri¹, Federico Fanti¹, Carmine Merola²,
Giampiero Scortichini³, Michele Del Carlo¹, Dario Compagnone¹**

¹ Dipartimento di Bioscienze e Tecnologie Agro-Alimentari ed Ambientali, Università degli Studi di Teramo, 64100, Teramo, Italy

² Dipartimento di Medicina Veterinaria, Università degli Studi di Teramo, 64100, Teramo, Italy

³ Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise G. Caporale, 64100, Teramo, Italy

Keywords: *Pendimethalin, Zebrafish, UHPLC-HRMS, Metabolism*

Pendimethalin is a dinitroaniline herbicide widely applied for the pre-emergence control of annual grasses and broadleaf weeds. Its low water solubility, marked lipophilicity, and environmental persistence contribute to its bioaccumulation potential in non-target organisms [1]. Although its toxicological profile has been partially investigated, the metabolic fate of pendimethalin in vertebrates remains insufficiently characterized, with most available studies focusing on mammalian excretion rather than comprehensive metabolite profiling in aquatic species [1,2]. Pendimethalin frequently reaches aquatic environments through agricultural runoff, resulting in chronic exposure of fish to environmentally relevant sub-lethal concentrations. Importantly, metabolites may exhibit persistence and toxicity profiles distinct from those of the parent compound; however, their chemical identity and biotransformation pathways in aquatic vertebrates remain largely unexplored. Zebrafish (*Danio rerio*) represents a well-established ecotoxicological model for investigating xenobiotic biotransformation in freshwater species [3]. In this work, we developed an integrated workflow combining *in silico* metabolic prediction with UHPLC-HRMS/MS analysis for the structural characterization of pendimethalin metabolites in zebrafish exposed under controlled laboratory conditions. Metabolic prediction software was employed to generate a high-confidence inclusion list encompassing expected phase I biotransformations (oxidation, aromatic hydroxylation, N-dealkylation, nitro reduction) as well as potential phase II conjugation products. Analyses were performed using an Orbitrap IQ-X platform operating in targeted MS/MS acquisition mode at high resolving power. Structural annotation was achieved through systematic comparison of the fragmentation spectra of detected features with those of the parent compound, employing a computational approach by a systematic Python pipeline, evaluating diagnostic fragment conservation, characteristic neutral losses, and accurate mass shifts consistent with the predicted biotransformations. This strategy enabled the detection of previously unreported putative pendimethalin metabolites in zebrafish, supported by mass accuracy <5 ppm and consistent MS/MS fragmentation behaviour. Overall, the study demonstrates the effectiveness of a prediction-driven HRMS approach for elucidating biotransformation pathways of environmentally persistent herbicides in aquatic vertebrate models.

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PFAS an analytical emergency finally solved?

Emanuele Ceccon¹

¹ Restek Italia s.r.l Cernusco sul naviglio, Italy

While we started testing PFAS in just water, today we test a wide variety of different matrices, each with its own regulations, targets and limits.

What do we mean when we talk about PFAS? Which technologies are involved in PFAS analysis other than LC and GC? Which instruments operate at the proper sensitivity?

Your mass detector is the starting point to understanding which analytical approach you should pursue. Here follows a practical approach guideline for PFAS analysis, basing the approach on your detector sensitivity.

Results

Once you know your instrument, you have all the information you need to start solving the PFAS emergency at analytical level.

A single run to analyze all PFAS together? Separate runs to maximize sensitivity? Whatever your best practice, there's a way to make it happen.

Discussion and conclusions

How can we easily develop an analytical method for PFAS? We'll discuss what is needed to evaluate your analytical approach, based on the sensitivity of your MS detector.

Do you own an older instrument or a high-resolution mass spectrometer? Then you'll need a different approach compared to people who are using a latest-generation triple quadrupole. We'll discuss both approaches, outlining the reasons behind our choices, ensuring that your analysis allows you to reach the required sensitivity.

What about PFAS that are best suited to analysis by GC? We're well-prepared for this as well, and we'll show you our most advanced PFAS GC method.

In addition to turnkey methods, Restek offers the EZ suite, a comprehensive free method development tools dedicated to the PFAS world. This means that you can create or modify your own method with your own list of analytes in just over 5 minutes at the computer before actually going to the instrument, saving you both time and expense.

Validation of an Enhanced GC–MS Isotope Dilution Method for PFAS Determination in Water

Daniele Zatta¹, Davide Reiss Balbo^{1,2}, Elena Rizzo¹, Luca Cappellin¹

¹ *Department of Chemical Sciences, University of Padua, Via Marzolo 1, 35131 Padua, Italy*

² *LA.RI.AN. IDROPUR, Via Rovigo, 77, 35042 Este (Padua), Italy*

Keywords: PFAS, GC-MS

Per- and polyfluoroalkyl substances (PFAS) are a large class of persistent organofluorine compounds widely used in industrial and consumer applications. Due to their extreme environmental persistence, and documented adverse health effects, PFAS are subject to increasingly stringent international regulations. In the European Union, Directive (EU) 2020/2184 establishes parametric values for PFAS in drinking water, including limits for PFAS Total (0.50 µg/L) and Sum of PFAS (0.10 µg/L), while some Member States, such as Italy, have implemented even stricter requirements, assigning 0.02 µg/L to the sum of PFOA, PFOS, PFNA and PFHxS [1,2].

Liquid chromatography – tandem mass spectrometry (LC–MS/MS) is currently the reference technique for PFAS determination in water [3]. However, gas chromatography – mass spectrometry (GC–MS), when combined with appropriate derivatization strategies, could represent an alternative approach that can offer advantages in terms of chromatographic resolution, selectivity, and accessibility. GC-based methods employing in-injector derivatization with diaryliodonium salts have been previously reported for PFAS analysis, but their application has so far been limited in compound coverage and regulatory validation [4,5].

In this work, we present a significantly improved GC–MS method based on in-injector diaryliodonium derivatization combined with isotope dilution for the determination of PFAS in potable water. The method was expanded to include 30 PFAS of regulatory relevance. Isotopically labelled internal standards were employed to correct for derivatization efficiency, matrix effects, and instrumental variability.

A comprehensive validation was carried out in accordance with regulatory expectations for drinking-water analysis, including assessment of selectivity, linearity, limits of detection and quantification, trueness, precision, and robustness. Method performance was evaluated at concentration levels relevant to compliance monitoring, and applicability was demonstrated through the analysis of real drinking-water and wastewater samples. The validated method achieves limits of quantification compatible with current regulatory thresholds and provides reproducible and accurate results across the full target list. This study demonstrates that GC–MS with in-injector derivatization can serve as a reliable and complementary alternative to LC–MS/MS for PFAS compliance monitoring in water.

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Linking lipid metabolism to the immune landscape of clear cell renal cell carcinoma through MS-driven spatial multi omics

Greta Bindi¹, Federico Piazza¹, Vasco Coelho¹, Sara Ghirardi¹, Angela Greco², Vincenzo L'Imperio², Fabio Pagni², Fulvio Magni¹ and Andrew Smith¹

¹ *Unit of Proteomics and Metabolomics, Department of Medicine and Surgery, University of Milano-Bicocca, 20854, Veduggio al Lambro, MB, Italy*

² *Unit of Pathology, IRCCS Fondazione San Gerardo dei Tintori, Via G. B. Pergolesi, 33, Monza, Italy*

Keywords: *Mass Spectrometry Imaging, Renal Carcinoma, Tumour-Immune Microenvironment, MALDI-HiPLEX-IHC, Spatial Lipidomics*

Clear cell renal cell carcinoma (ccRCC) represents the most frequent histological subtype of renal carcinoma and is characterized by marked immune infiltration and profound metabolic remodelling, particularly affecting lipid metabolism. Despite the significant presence of immune cells within the tumour microenvironment, the efficacy of immune checkpoint inhibitor-based therapies remains limited: only a subset of patients achieves durable clinical benefits, while many fail to respond or develop acquired resistance over time [1]. This highlights the complexity of the tumour microenvironment and underscores the need for a more comprehensive characterization of both the immune component and the metabolic alterations that may modulate the immune response, promote tumour progression, and contribute to therapeutic resistance.

In this context, spatially resolved approaches such as mass spectrometry imaging (MSI) represent a promising tool to investigate this complexity, as they enable the mapping of various biomolecules, including lipids, within the tumour microenvironment. Moreover, spatial lipidomics can be complemented by the integration of MALDI-HiPLEX-IHC, which enables targeted multiplexed mapping of protein antigens, on the same tissue slide [2], thus providing an additional layer of molecular information and enabling the correlation of specific immune cell populations and their influence on the surrounding metabolic environment in ccRCC.

Accordingly, this work focuses on the optimization of a multiomics workflow to link the immune landscape of ccRCC tumours, with particular attention to immune cell populations associated with tumour infiltration and the expression of immunosuppression-related markers, with their impact on the lipidomic composition of the tumour microenvironment, all achieved on a single tissue section. To accomplish this, open-source computational pathology tools are employed to generate masks that define the spatial distribution of immune cell subsets and enable the correlation with spatial lipidomic datasets obtained.

This work uniquely enables the highly multiplexed characterization of the immune milieu of ccRCC whilst highlighting lipidomic alterations associated with specific immune cell infiltration on the same tissue section. These findings may contribute to a better understanding of the complex metabolic interactions occurring within the tumour microenvironment of these highly infiltrated tumours.

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Proteomic characterization of CAR-T cell-derived extracellular vesicles as a potential cell-free strategy for cancer immunotherapy

Alice Di Sebastiano¹, Maria Concetta Cufaro¹, Francesco Guardalupi², Francesca D'Ascanio¹, Serena Veschi³, Serena Pilato³, Giulia Corradi¹, Damiana Pieragostino¹, Mauro Di Ianni², Paola Lanuti¹ and Piero Del Boccio¹

¹ Center for Advanced Studies and Technology, University "G. d'Annunzio" of Chieti-Pescara, Italy

² Department of Medicine and Aging Sciences, University "G. d'Annunzio" of Chieti-Pescara, Italy

³ Department of Pharmacy, University "G. d'Annunzio" of Chieti-Pescara, Italy

Keywords: CAR-T cells, antineoplastic therapy, extracellular vesicles, label-free proteomic

Chimeric antigen receptor (CAR)-T lymphocytes represent the most innovative form of immunotherapy against cancer and have revolutionized cancer treatment of hematological malignancies. However, despite their successes, many challenges remain to be addressed, including their severe adverse effects, such as cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) [1]. Extracellular Vesicles (EVs) are membrane-enclosed vesicles released by all cell types, carrying a biomolecular cargo (consisting of proteins, mRNA, and microRNAs) driving a massive intercellular crosstalk [2]. CAR-T cell-derived EVs potentially mimic the anti-tumor activity of their parental cells, limiting cell associated toxicity. In this study, we applied a shotgun label-free mass spectrometry-based proteomics workflow to characterize the protein profile of CD19.CAR-T cell-derived EVs. EVs were isolated by a novel developed method based on Fluorescence Activated Cell-Sorting (FACS) able to purify specific subtyping of EVs using recognition strategy based on antibody-antigen reaction. In our study, we applied this method to purify and characterize CD19.CAR+EVs from both blood of patients treated with commercial CD19.CAR-T cells and from commercial CAR-T bags. For proteomic analysis, approximately 1×10^6 sorted EVs were collected for each sample. To maximize protein recovery from low-input vesicular material for downstream LC-MS/MS analysis, the purified vesicle samples were processed using an optimized SP3-based sample preparation protocol. Morphological analysis confirmed that CD19.CAR+EVs had a mean diameter of less than 200 nm, classifying them as small EVs. Proteomic analysis revealed that 97.17% of the proteins from circulating and pre-infusion CD19.CAR+EVs were shared and most of them are involved in pro-apoptotic pathways confirmed by in vitro killing assays. Moreover, A deeper proteomics analysis of EVs derived from different CAR-T cell subpopulations including those expressing exhaustion markers such as PD-1 and LAG-3, identified a pro-apoptotic functional pathway linked to CD8+LAG-3+ EVs. Overall, our data suggest that CD19.CAR+EVs may represent promising alternative product with a therapeutic potential that could be infused independently from CAR-T cells.

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Advancing Thyroid Nodule Diagnosis: Integrating MALDI-MSI for Detailed Characterization of Indeterminate Lesions

**Nicole Monza¹, Felisia Di Nicoli¹, Marta Nesteruk², Vasco Coelho¹, Vincenzo L'Imperio³,
Claudia Fumagalli¹, Lisa Pagani¹, Andrew Smith¹, Fabio Pagni³, Fulvio Magni¹, Giulia Capitoli²
and Vanna Denti¹**

¹ *Proteomics and Metabolomics Unit, Department of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy*

² *Bicocca Bioinformatics Biostatistics and Bioimaging B4 Center, School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy*

³ *Department of Medicine and Surgery, Pathology, University of Milano-Bicocca, IRCCS Fondazione San Gerardo dei Tintori, Monza, Italy*

Keywords: *Thyroid Cancer, MALDI-MSI, proteomics*

The accurate diagnosis of premalignant lesions, such as Non-Invasive Follicular Thyroid Neoplasm with Papillary-like Nuclear Features (NIFTPs), remains challenging due to its histological similarities to both benign and malignant follicular thyroid lesions [1]. Differentiating NIFTP premalignant lesions from the malignant ones is fundamental to avoid unnecessary overtreatment [2, 3]. This study was conducted on a large cohort comprising seven formalin-fixed paraffin-embedded (FFPE) tissue microarrays (TMAs), including a total of 332 cores from 183 patients. The cohort encompassed a spectrum of thyroid lesions, including follicular adenoma (FA), Hürthle cell adenoma (HA), NIFTP, follicular variant papillary thyroid carcinoma (FVPTC), and papillary thyroid carcinoma (PTC). For each lesion, cell-rich regions were selected using a pixel classifier algorithm implemented in QuPath software, excluding confounding areas such as collagen, stroma, and artefacts, thereby enabling robust management of this large dataset [4]. To investigate the proteomic landscape of thyroid lesions, we combined trypsin-digested spatial proteomics based on matrix-assisted laser desorption/ionisation (MALDI) mass spectrometry imaging (MSI), acquired with a timsTOF flex instrument (Bruker) at 20 µm spatial resolution, and an advanced biostatistical modelling framework. This integrative approach was designed to improve the molecular characterisation of premalignant lesions by capturing distinct aspects of the underlying signal structure. Preliminary analyses performed using MetaboAnalyst identified a panel of 15 discriminative m/z features that distinguish NIFTP from other lesion types, corresponding to proteins involved in proliferative signalling. Using a support vector machine (SVM) model, we achieved an accuracy close to 90%. Currently, network-based statistical methods are applied on the same dataset to further validate the results. These advanced statistical approaches enable the detection of differences arising from underlying dependency structures among variables, and not solely from individual feature effects, extracted from MALDI-MSI data. Overall, the integration of spatial proteomic analysis with advanced biostatistical tools opens new perspectives for a more accurate diagnosis and classification of NIFTPs, representing an important step forward in the diagnosis and potentially treatment of thyroid neoplasms.

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Phospho-proteomic profiling of Calu3 cells infected with SARS-CoV-2 reveals Bcl2-associated athanogene 3 (BAG3) phosphorylation and AKT/MAPK signaling activation

Federica Di Marco¹, Fabio Di Ferdinando¹, Erika Pizzinato¹, Beatrice Dufrusine², Ilaria Cicalini¹, Margot De Marco³, Alessandra Rosati³, Maria Caterina Turco³, Piero Del Boccio⁴, Vincenzo De Laurenzi¹ and Damiana Pieragostino¹

¹ Centre for Advanced Studies and Technology, University "G. d'Annunzio" of Chieti-Pescara

² Department of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo

³ Department of Medicine, Surgery and Dentistry "Schola Medica Salernitana", University of Salerno

⁴ Department of Sciences, University "G. d'Annunzio" of Chieti-Pescara

Keywords: SARS-CoV-2, BAG3, IFITM, phosphorylation cascade, phospho-proteomics

Although the secreted role of BAG3 has been extensively characterized in cancer, where it promotes tumor progression via interaction with interferon-induced transmembrane protein 2 (IFITM2) and stimulation of cytokine release, its involvement in viral infections remains poorly understood [1,2]. Our previous data indicate that BAG3 is also secreted by SARS-CoV-2-infected Calu3 cells, while IFITM2 has recently been described as a critical co-receptor for viral entry and replication; however, the molecular mechanisms underlying this interaction remain unclear [3]. To dissect phosphorylation-driven signaling and infection-associated events, we implemented a label-free shotgun phospho-proteomics workflow optimized for sensitivity and phosphosite localization. Proteins were extracted from uninfected (CC) and SARS-CoV-2 infected (CI) Calu-3 cells and processed by SP3-based digestion protocol, ensuring efficient cleanup and reproducibility. Phosphopeptides were selectively enriched using TiO₂ beads and analyzed by high-resolution nano-LC coupled to Orbitrap MS/MS, enabling deep coverage of the phosphoproteome. Raw data were processed by Proteome Discoverer with integrated phospho-site localization scoring, followed by functional bioinformatics analysis on STRING and Ingenuity Pathway Analysis (IPA). Quantitative analysis identified 3,208 phosphopeptides corresponding to 1,009 robustly quantified phosphoproteins, revealing extensive infection-associated remodeling. Notably, BAG3 emerged as an infection-related phosphoprotein, with five confidently localized phosphorylation sites (S173, T285, S289, S377, S386). Many of these sites are reported in oncogenic contexts, while their functional role in viral infection and IFITM2 interaction remains unexplored. Differential phospho-signatures were strongly associated with SARS-CoV-2-related pathways and host-virus interaction processes, highlighting phosphorylation as a key regulatory layer. Upstream regulator analysis indicated activation of AKT1 and MAPK1 signaling cascades, consistent with modulated viral replication networks observed in infected cells. Overall, this mass spectrometry-driven phospho-proteomic approach provides a comprehensive view of host signaling rewiring upon SARS-CoV-2 infection, identifying BAG3 and AKT/MAPK pathways as central nodes and underscoring the value of advanced phospho-proteomics workflows in studying virus-host interactions.

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In-source fragmentation of oxysterols: from analytical pitfalls to confident identification by high-resolution mass spectrometry

Andrea Castellaneta¹, Ilario Losito^{1,2}, Martina Solarino¹, Vito Nettis¹, Cosima Damiana Calvano^{1,2}, Tommaso R.I. Cataldi^{1,2}

¹ Dipartimento di Chimica, Università degli Studi di Bari Aldo Moro, 70126 Bari, Italy

² Centro Interdipartimentale SMART, Università degli Studi di Bari Aldo Moro, 70126 Bari, Italy

Keywords: Oxysterols, in-source fragmentation, high-resolution mass spectrometry

Oxysterols (OSs) are a broad class of compounds generated by the oxidation of cholesterol and its biosynthetic precursors. Beyond their role as intermediates in bile acid and steroid hormone biosynthesis, OSs act as signalling molecules and are key regulators of cholesterol homeostasis [1]. Because they are typically present at trace levels in biological matrices, their analysis requires highly sensitive analytical approaches. For this reason, mass spectrometry (MS)-based detection is considered the gold standard for OS characterisation. Soft atmospheric-pressure ionisation techniques have enabled the direct coupling of liquid chromatography with MS, overcoming several limitations of derivatisation-based strategies traditionally used to increase analyte volatility for gas chromatography. In LC-MS workflows, chemical derivatisation may still be employed to enhance ionisation efficiency. However, strict control of the derivatisation process requires deuterated analogues to monitor reaction yields, which are not always available. Conversely, when derivatisation is avoided, sensitivity may be compromised if the intrinsic tendency of OSs to undergo extensive in-source fragmentation is not properly considered. This issue can be particularly limiting in MS methods based on selected precursor ions, such as multiple-reaction monitoring (MRM). In this context, a comprehensive understanding of the ion channels across which the ion population is distributed is essential to improve sensitivity, especially when selectivity is provided by high-resolution mass spectrometry (HRMS). The present work, therefore, investigates, in a systematic manner, the in-source fragmentation patterns induced by the distinctive oxygen-containing functional groups introduced during oxidation of the cholesterol backbone. Rationalising these fragmentation pathways reveals potential pitfalls related to the formation of isomeric ions from non-isomeric species. At the same time, kinetic and thermodynamic features underlying specific fragmentation behaviours may support the differentiation of OS isomers at the MS1 level, based on the relative signal intensities of shared ionisation channels. Overall, in-source fragmentation patterns can be regarded as molecular signatures useful for tracking OS peaks during LC method development [2]. They may also provide orthogonal confirmation to retention time, ideally without requiring additional tandem MS events [3], thereby increasing acquisition frequency. This feature is particularly relevant when HRMS detection is combined with ultra-high-performance liquid chromatography, where narrow chromatographic peaks demand fast and information-rich acquisition strategies.

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Untargeted UHPLC-HRMS Metabolomics of Water Kefir: Effect of Different Substrates on Metabolic Profiles

Sara Palmieri¹, Fabiola Eugelio¹, Marcela Isabel Rico Benitez², Marinella Tano¹, Francesca Dell'Orco¹, Federico Fanti¹, Roberta Prete¹, Aldo Corsetti¹, Ruth Elizabeth Belmares Cerda², Michele Del Carlo¹, Dario Compagnone¹

¹ *Dipartimento di Bioscienze e Tecnologie Agro-Alimentari ed Ambientali, Università degli Studi di Teramo, 64100, Teramo, Italy*

² *Food Research Department Autonomous University of Coahuila, Saltillo, Coahuila, Mexico*

Keywords: *Water kefir fermentation, Untargeted metabolomics, UHPLC-HRMS, Spectral annotation, Molecular networking*

Water kefir is a plant-based fermented beverage whose relatively simple, yet interaction-rich microbial community has recently attracted growing interest as a model system for untargeted metabolomics and integrated multi-omics investigations [1,2]. Pan multi-omics studies have revealed marked temporal shifts in both microbial composition and metabolite space over the course of fermentation, with substrate composition emerging as a key driver of community succession dynamics and metabolic output [2,3]. Despite this, the chemical consequences of using structurally distinct carbohydrate sources, such as honey, rich in bioactive phytochemicals and organic acids, versus refined cane sugar, remain poorly characterized at the molecular level, particularly when water kefir grains are used as inoculum. Untargeted Ultra-High Performance Liquid Chromatography - High Resolution Mass Spectrometry (UHPLC-HRMS) metabolomics, combined with advanced spectral annotation tools, offers a powerful approach to resolve these substrate-specific biotransformation patterns across fermentation time. In this study, water kefir, prepared using water kefir grains as inoculum, was characterized using both wildflower honey and brown sugar as the fermentation matrix. Three independent batch fermentations per matrix were sampled at T0, T24, T48, and T72 h, with pooled QC samples included throughout. Sample preparation was optimized against literature methods. Data were acquired in positive and negative Electrospray Ionization (ESI) mode using full scan coupled with data-dependent MS/MS acquisition (DDA) at <5 ppm mass accuracy (Orbitrap IQ-X, Thermo Scientific). After QC-based normalization using Systematic Error Removal using Random Forest (SERRF), differential analysis across consecutive timepoints identified 609 and 877 significant features in honey and sugar, respectively. Spectral molecular networking combined with SIRIUS/CSI: FingerID annotation and CANOPUS class prediction yielded 574 and 778 annotated structures. The two matrices showed divergent metabolite profiles: honey fermentation was enriched in amino acids, peptides, and organic acids, while sugar fermentation was dominated by shikimate-derived metabolites and carbohydrates, highlighting distinct substrate-specific biotransformation pathways.

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Breaking the 6-Second Barrier: Evaluating the Innovative DBDI-MS Tool for Rapid Foodomics Analysis

Carmela Zacometti¹, Andrea Massaro¹, Francesca Asson¹, Severino Segato², Alessandra Tata¹

¹ Istituto Zooprofilattico Sperimentale delle Venezie, Laboratorio di Chimica Sperimentale Viale Fiume, 78, Vicenza Italy

² Department of Animal Medicine, Production and Health, University of Padova, Italy

Keywords: ambient mass spectrometry, DBDI-MS, SICRIT-MS, authenticity

In our search for a rapid and cost-effective tool to determine the chemical composition of food, we examined the analytical capabilities of dielectric barrier discharge mass spectrometry (DBDI-MS). We evaluated its ability to characterize honey, grape pomace, and cheese, which have very different compositions and textures. For the DBDI-HRMS analysis, we used a SICRIT® source (Plasmion) coupled to an Exactive Orbitrap (Thermo Fisher). DBDI-HRMS is characterized by a high-voltage alternating current that generates a cold plasma from environmental molecules. This plasma then ionizes the volatile compounds, which are drawn into the ultra-high vacuum of the MS. After adapting the extraction procedure slightly for each food (adding or omitting solvent and salt) and mildly heating the samples, we analyzed them by placing the open container directly in front of the sampling source for six seconds. Statistical analysis was conducted to allow pattern recognition. We used the MALDIquant package of the R 4.3.2 software and the MetaboAnalyst 5.0 web portal for this purpose. Nonparametric ANOVA (analysis of variance) ($p\text{-value adj.} \leq 0.05$) and principal component analysis (PCA) were employed to interpret the results effectively. Machine learning algorithms were used for classification purposes. Initially, DBDI-HRMS revealed molecular features that uniquely characterize the floral sources of the seven monofloral honey types studied. When used for machine learning and automatic honey authentication, DBDI-HRMS data achieved excellent performance in terms of accuracy, area under the curve, sensitivity, and specificity. These methods can be considered reliable for determining honey's botanical origin. Moreover, the grape waste was used as an ingredient in goats feed in order to verify if this supplementation has an impact on the animal and, more importantly, on the resulting cheese product. In this case, DBDI-HRMS was employed for the assessment of aromatic compounds in order to retrieve the aromatic profile of grape pomace and the resulting goat cheese. It was found that cheeses derived from goats fed with grape supplements showed significant differences with the control group. Specifically, higher levels of certain aldehydes and ketones. In addition to the VOCs variations in goat cheese produced from various diets, the maturation process clearly led to the evolution of the volatile organic compounds. The differences observed in the volatile profiles of cheeses from grape pomace-fed animals compared with control cheeses were attributed to the polyphenol-rich composition of grape pomace and its potential antioxidant influence on oxidative, microbial, and enzymatic processes during ripening. DBDI-HRMS method could rapidly interrogate the samples, capturing the important and distinctive compounds in the aroma of honeys within 6 s, thus saving time and resources and allowing more efficient use of laboratory equipment. Careful late-stage validation of this ambient mass spectrometry method, including repeatability checks, are mandatory to move these methods into industrial quality controls. In conclusion, using this plasma-based technique, the volatile compounds released in just seconds by opening the lid of the tube of thermally treated cheese were acquired. The key features of the setup are cold plasma-based ionization together with high resolution mass spectrometry that operates effectively with a relatively small amount of volatile compounds. We showed that DBDI-HRMS unveils various odor active compounds that distinctly characterize honey, grape by-products and cheese.

Monitoring of Reactive Carbonyl Compounds in Food Matrices via SPME-GC-MS/MS

Mauro Paolini¹, Roberto Larcher¹

¹ *Fondazione Edmund Mach, 38098 San Michele all'Adige, Italy*

Keywords: Aldehydes, PFBHA derivatization, HS-SPME-GC-MS/MS

Characterizing the volatile profile of thermally treated or aged food matrices is challenging due to the high reactivity of carbonyl compounds. Aldehydes are ideal chemical markers for Strecker degradation and the Maillard reaction. However, their chemical instability makes their analytical determination highly complex. Key molecules such as methional, phenylacetaldehyde, furfural, and branched aldehydes have extremely low olfactory thresholds, often in the ng/L range. Therefore, quantifying them at sensorially relevant levels requires highly sensitive methods with low limits of detection. To stabilize these analytes and prevent evaporative loss or degradation, an optimized protocol was developed. It combines in-situ derivatization using O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBHA) with headspace solid-phase microextraction and gas chromatography-tandem mass spectrometry (HS-SPME-GC-MS/MS). Operating the triple quadrupole (QqQ) in Multiple Reaction Monitoring (MRM) mode proved crucial to enhance analytical selectivity. The optimization of collision-induced dissociation parameters allowed for the consistent isolation of diagnostic fragments common to all PFB-oxime derivatives. These include the highly intense pentafluorobenzyl cation $[C_7H_2F_5]^+$ (m/z 181) and the tetrafluorobenzyl cation $[C_7HF_4]^+$ (m/z 161) (resulting from the neutral loss of HF), which facilitate peak detection through retention time alignment. To ensure unambiguous identification, these universal transitions were coupled with analyte-specific transitions of lower intensity, related to the parent aldehyde structure. This approach effectively suppressed chemical background noise and compensated for the matrix effect in highly heterogeneous samples. The method's versatility was preliminarily tested in the baking sector to monitor aldehyde evolution under varying baking conditions. Furthermore, in oenology, this procedure enables accurate quantification of the total aldehyde pool. This accuracy is vital for assessing a wine's aromatic potential, as the free aldehyde fraction responsible for sensory perception is heavily modulated by sulfur dioxide equilibrium and the formation of non-volatile hydroxysulfonate adducts. Ultimately, this robust MS-based approach proves essential for understanding reaction mechanisms and optimizing food production chains, allowing for the precise quantification of reactive analytes that would otherwise remain elusive.

Mapping drought-driven metabolic remodelling in coffee fine roots by MALDI-MS imaging

Sara Ghirardi¹, Greta Bindi¹, Paola Crisafulli², Fulvio Magni¹, Luciano Navarini², Andrew Smith¹

¹ University of Milano-Bicocca, Unit of Proteomics and Metabolomics, Department of Medicine and Surgery, Vedano al Lambro, via Raoul Follereau 3, Vedano al Lambro 20854, Italy

² Illycaffè S.p.A., via Flavia 110, Trieste I-34147, Italy

Keywords: MALDI-MSI, spatial metabolomics, *Coffea canephora*, Mass spectrometry imaging, BOLERO project

The breeding of fruit tree crops, such as coffee, is threatened by challenging climate conditions, including those induced by climate change, such as drought. Based on these premises, in 2022, the European Union funded the BOLERO project “to create resilient rootstock varieties for fruit tree crops to cope with climate change threats” (www.bolero-project.eu). In this work, we employed MALDI-MSI to map the metabolic alterations occurring in the fine roots of the *Coffea canephora* species following drought conditions to address the BOLERO project’s objective to “understand root-based traits”. A total of 40 root sections, 20 from roots exposed to water-stressed (WS) and 20 well-watered controls (WW), were cut at a thickness of 20 µm and either Norharmane or 6-aza-thiothymine (ATT) were deposited using the HTX TM Sprayer™ M3. All imaging analyses were performed with a Bruker timsTOF fleX™ instrument operating in negative-ion mode within the m/z 50–1000 range and utilising a lateral resolution of 20 µm. Subsequently, all sections were stained with toluidine blue in order to annotate the epidermis/exodermis, cortex, and secondary xylem of each root. Metabolic alterations between the WS and WW roots were observed within all three of the primary tissues; however, the most notable adaptations were observed within the epidermis/exodermis regions that are more greatly exposed to external environmental conditions. A general decrease in metabolic activity was noted, as well as a distinct metabolic reprogramming in WS roots. In particular, an increase in metabolites related to energy metabolism (e.g., fumaric acid, malic acid) [1] and the biosynthesis of defensive secondary metabolites (e.g., umbelliferone) was detected [2]. Conversely, a decrease in a subset of detected ions may correspond to lignin-related compounds or their degradation products (e.g., syringin), potentially reflecting structural adaptations of the cell wall under drought stress, although these assignments require further validation; this, despite speculations, may reflect a weakening of these regions, a hypothesis supported by the increased presence of lacunae observed in the WS group. In parallel, MS/MS experiments are in progress to support peak annotation. These preliminary results represent the contribution that spatial metabolic profiling can provide when seeking to better understand the metabolic adaptations occurring in *Coffea* roots following exposure to drought and how these may be targeted to render such crops more capable of coping with climate change threats.

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Formation of Copper(II) Oxide Nanoparticles by ESI Microdroplets Deposition

Lorenzo D'Ippolito¹, Chiara Salvitti¹, Anna Troiani¹, Marta Managò¹, Alessia Di Noi¹, Franco Mazzei¹, Marco Agostini¹, Rosaceleste Zumpano¹, Isabella Chiarotto², Andreina Ricci³ and Federico Pepi¹

¹ Department of Chemistry and Technology of Drugs, Sapienza University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy

² Department of Basic and Applied Sciences for Engineering, Sapienza University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy

³ Department of Mathematics and Physics, University of Campania L. Vanvitelli, Viale Lincoln 5, 81100, Caserta, Italy

Keywords: copper(II) oxide nanoparticles, ESI microdroplets deposition, reaction acceleration

The utilization of electrospray ionization (ESI) source from a mass spectrometer to generate charged microdroplets represents a cutting-edge strategy for inducing accelerated chemical transformations in confined volumes [1]. These microdroplets act as extremely dynamic microreactors, defined by instantaneous solvent evaporation, intense interfacial electric fields, and sudden pH variations. Such conditions allow for reaction rates up to six orders of magnitude higher than those of the same processes conducted in bulk phase [2-3]. Recently, copper(II) oxide nanoparticles (CuONPs) have been adopted as significant materials for catalysis, sensing, antimicrobial coatings, and energy storage; consequently, identifying alternative synthetic pathways is essential to promote the widespread application of these materials. This study evaluates the feasibility of synthesizing CuO nanoparticles through the deposition of ESI microdroplets from aqueous copper(II) acetate solutions under mild conditions, avoiding the strongly alkaline environments typical of traditional wet-chemical methods. Slightly acidic Cu(AcO)₂ aqueous solutions were nebulized via a Z-spray ESI source to produce charged droplets directed onto solid substrates, where their coalescence originated reactive thin films. The evolution of clusters and ionic species was monitored via ESI mass spectrometry, while the obtained deposits were analyzed using scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), and X-ray photoelectron spectroscopy (XPS). The effects of deposition time and desolvation conditions were examined to optimize particle production. ESI-MS analyses highlighted the formation of copper-acetate clusters containing CuO units already during the microdroplets flight, suggesting that conversion begin within the confined environment of the droplet. CID analyses show a consistent loss of CuO centers, further confirming the proposed mass assignments. Prolonged deposition times generated solid films from which the CuO was selectively isolated following the removal of soluble precursors. The application of a negative voltage to the ESI source proved decisive in favoring CuO formation, whereas positive or zero potentials facilitated the formation of metallic copper.

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The curious case of TEMPO-photocatalysed Knoevenagel condensation: Unveiling hidden reaction pathways through MS monitoring

Chiara Salvitti¹, Alessia Di Noi¹, Lorenzo D'Ippolito¹, Federico Pepi¹, Isabella Chiarotto², Anna Troiani¹

¹ *Dipartimento di Chimica e Tecnologie del Farmaco, Sapienza Università di Roma, 00185 Roma, Italy*

² *Dipartimento di Scienze di Base e Applicate per l'Ingegneria, Sapienza Università di Roma, 00185 Roma, Italy*

Keywords: *Knoevenagel condensation, TEMPO radical, MS monitoring, reaction mechanisms*

The Knoevenagel condensation is a well-known chemical reaction between compounds with an active methylene group (*e.g.* malononitrile, ethyl cyanoacetate) and aldehydes (or ketones) leading to the formation of alpha-(beta) unsaturated condensation products [1]. Due to its broad application in the synthesis of different compounds, such as natural products, pharmaceuticals, and polymers [2], the search for alternative catalysts beyond traditional bases remains an active and growing area of research. In this framework, the expanding demand for greener synthetic methods has encouraged the adoption of water or ionic liquids as alternative solvents [3], as well as the use of nanomaterial catalysts, such as carbon dots (CDs) [4].

The stable 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO) is a nitroxyl compound commonly used as a free radical scavenger to probe radical-mediated reaction pathways [5]. Owing to its redox properties, TEMPO can also act directly as a catalyst, for example in the dehydrogenation of activated alkanes [6] or in combination with copper salts for the aerobic oxidation of alcohols under green conditions [7]. Moreover, TEMPO was probed as a thermally activated tetramethylpiperidine (TMP) generator for the initiation of thiol-Michael addition polymerizations, laying the foundation for the design of more selective catalysts [8].

In this study, we investigated the photo-generation of TMP from TEMPO catalysed by dicarbonyl compounds with an acidic CH₂ carbon acting both as reaction initiator and substrate of the Knoevenagel condensation. The discovery of this catalytic system represents an intriguing case of serendipity, in which the high sensitivity of mass spectrometry used to monitor chemical reactions and detect elusive intermediates enabled the elucidation of an unprecedented catalytic role of TEMPO in the Knoevenagel condensation.

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A Multi-Platform Untargeted LC–HRMS Workflow Highlights Metabolic Differences Between *Brassica oleracea* Microgreens and Vegetative Plants

Francesca Monzillo¹, Luigi d'Aquino², Brigida Della Mura³, Sonia Piacente¹, Paola Montoro¹

¹ Department of Pharmacy, University of Salerno, via Giovanni Paolo II n. 132, 84084 Fisciano (SA), Italy

² Italian National Agency for New Technologies Energy and Sustainable Economic Development (ENEA), Portici Research Centre, Piazzale E. Fermi.1, 80055 Portici, Italy

³ Department of Science, University of Basilicata, Viale dell' Ateneo Lucano 10, 85100 Potenza, Italy

Keywords: microgreens, *Brassica oleracea*, precision agriculture, glucosinolates, LC–HRMS

Microgreens are nutrient rich functional foods with a rapidly expanding market relevance. However, their stage-dependent metabolic reprogramming remains poorly characterized. This study provides a comprehensive metabolomic comparison between microgreens and vegetative plants of *Brassica oleracea* var. *italica* (Brassicaceae), a species of particular interest for its glucosinolate (GLSs) content. The plants were cultivated under controlled conditions using a system integrating precision lighting and targeted ventilation in order to minimize variability in the metabolome due to environmental factors and ensure reliable and reproducible results [1]. An untargeted liquid chromatography–high resolution mass spectrometry (LC–HRMS) approach was applied to the plant extracts. Metabolite annotation was performed using a multi-platform workflow combining Compound Discoverer software with public spectral libraries (MassBank, METLIN, FooDB), taxonomically informed databases (LOTUS, KNApSACk), and computational MS/MS-based tools, integrating molecular formula determination and fragmentation tree-based fingerprint prediction (SIRIUS). Putative metabolite identifications were assigned at MSI level 2 confidence. Multivariate analysis (PCA and PLS-DA), validated by cross-validation and permutation testing, together with volcano plot analysis, revealed a pronounced metabolic divergence between the plant phenological stages. Glucosinolates emerged as the dominant discriminant class, being markedly enriched in microgreens and reflecting a dynamic remodelling of secondary metabolism during plant development. Overall, these findings demonstrate that microgreens represent a metabolically distinct and glucosinolate-enriched growth stage, with important implications for crop optimization and nutraceutical valorization.

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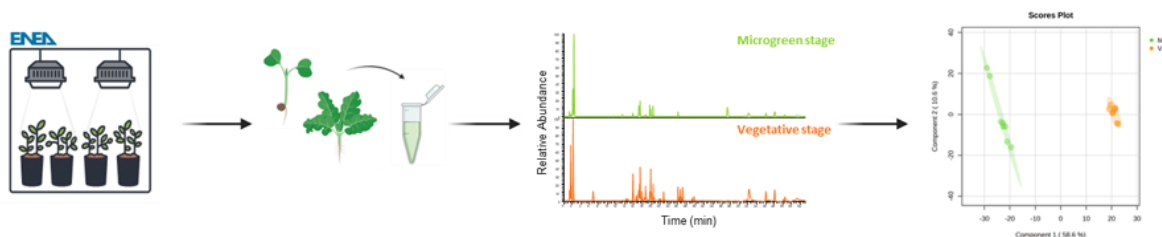


Figure 1. From controlled-environment plants cultivation to untargeted metabolomic profiling of microgreens and vegetative-stage broccoli extracts.

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Advanced data mining for Oxylipin characterization by LC-HRMS

Francesco Bartolini¹, Sandra M. Camunas-Alberca², Hana Cermakova², Ángel Sánchez-Illana³, David Perez-Guaita³, Pablo Cañadas-Miquel⁴, Alberto Gil-de-la-Fuente², Jean-Marie Galano⁵, Thierry Durand⁵, Ana Gradillas², Camilla Montesano¹, Manuel Sergi¹, Coral Barbas²

¹ *Sapienza University of Rome, Department of Chemistry, 00185, Rome, RM, Italy.*

² *Centro de Metabolómica y Bioanálisis (CEMBIO), Facultad de Farmacia, Universidad San Pablo-CEU, CEU Universities, 28660, Boadilla del Monte, Madrid, Spain*

³ *Department of Analytical Chemistry, University of Valencia, 46100 Burjassot, Valencia, Spain*

⁴ *Departamento de Tecnologías de la Información, Escuela Politécnica Superior, Universidad San Pablo-CEU, CEU Universities, 28660, Boadilla del Monte, Madrid, Spain*

⁵ *Institut des Biomolécules Max Mousseron (IBMM), UMR 5247, Pôle Chimie Balard Recherche, CNRS, Université de Montpellier, ENSCM, 34293, Montpellier, France*

Keywords: *Ion Identity Molecular Networking, Oxylipins, LC-HRMS*

Oxylipins are a class of bioactive lipid mediators produced through the oxidation of polyunsaturated fatty acids (PUFAs). In biological systems, they participate in essential physiological functions, including the control of blood circulation, intracellular signaling, and the regulation of inflammatory responses. However, their structural heterogeneity poses significant challenges for detection and annotation in untargeted LC-MS/MS analyses. In this work, we present the first application of the Ion Identity Molecular Networking (IIMN) approach to explore and organize the chemical diversity of oxylipins. We also provide a systematic evaluation of the main factors that limit reliable annotation in MS/MS datasets. Our approach leverages recent enhancements to the MZmine software, offering an alternative GNPS-independent method of connecting oxylipins in MN. We generated a comprehensive multidimensional dataset including oxylipin annotations, retention times, exact mass (m/z), and detailed information on adduct formation, cluster and in-source fragmentation profiles; Additionally, we constructed an MS/MS spectral library using 67 commercially available oxylipin standards, analyzed by LC-MS in data-dependent acquisition mode. A detailed characterization of the ion species formed during electrospray ionization, combined with the implementation of IIMN, enabled a significant reduction in network complexity. The modified cosine algorithm allows discrimination between full-length and cyclized species and supports the clustering of oxylipins based on structural coherence. This workflow was applied to mouse spleen extracts, integrated with both in-house and public MS/MS spectral libraries. It enabled the classification of oxylipins into coherent molecular families, supporting their structural annotation. These results highlight the efficiency of IIMN as a bioinformatics tool for the characterization of different compounds in complex biological samples.

Protein Secondary Structure Patterns in Short-Range Cross-Link Atlas

**Alice Vetrano^{1,2}, Alessio Di Ianni^{1,2}, Nico Di Fonte^{1,2}, Gianluca Dell'Orletta^{1,2}, Samantha Reale^{1,2},
Isabella Daidone^{1,2}, Claudio Iacobucci^{1,2}**

¹ *Department of Physical and Chemical Sciences, University of L'Aquila, Via Vetoio, L'Aquila 67100, Italy*

² *Human Technopole, V.le Rita Levi Montalcini 1, Milan 20157, Italy*

Keywords: *Chemical proteomics, Cross-linking, Mass spectrometry*

Cross-linking mass spectrometry (XL-MS) has become an essential tool in structural biology, as it provides valuable information on protein conformations and interactions. While long-range cross-links are widely used to define tertiary and quaternary structures, short-range cross-links (those spanning fewer than 20 residues) have often been overlooked and considered of little structural significance [1]. In this study, we systematically analyzed these short-range cross-links to explore their relationship with the secondary structure of proteins. We introduce X-SPAN, a software designed to integrate publicly available XL-MS datasets from system-wide experiments with AlphaFold-predicted protein structures for entire organismal proteomes. Specifically, we analyzed 12 publicly available system-wide XL-MS datasets, comprising a total of 658432 cross-links from 4 organisms. By applying our filtering criteria, selecting only unique intra-protein cross-links with a maximum spacing of 20 amino acids in the primary sequence, we identified 78985 cross-links, of which 48748 were found in continuous motifs (α -helices, β -strands coils and mixed elements).

Our analysis unveils distinct cross-linking patterns that depend on the spatial constraints of secondary structure elements, with α -helices, β -strands, and coils exhibiting characteristic behaviors. We demonstrate that α -helices display a periodic cross-linking pattern consistent with their helical pitch, whereas coils and β -strands show nearly monotonic distributions.

Additionally, our analysis highlights context-dependent cross-linking grammar, where the local amino acid environment modulates cross-linking efficiency.

By comparing experimental cross-linking data with AlphaFold-predicted structures, we find that cross-link distributions are strongly influenced by the predicted local confidence score (pLDDT). This work provides a novel framework for benchmarking AlphaFold's local accuracy.

Beyond these structural insights, our study demonstrates the potential of short-range cross-links as quality control metrics for XL-MS experiments to distinguish between native distributions from potential artifacts.

We anticipate that our short-range cross-link database will serve as a valuable resource for studying local secondary structure switches and their potential roles in protein function and allosteric regulation.

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Co-registration of mass spectrometry and optical whole-slide histology images for computational pathology

Vasco Coelho¹, Nicole Monza¹, Natalia S. Porto¹, Claudia Fumagalli¹, Andrew Smith², Vincenzo L'Imperio², Vanna Denti²

¹ *Proteomics and Metabolomics Unit, Department of Medicine and Surgery, University of Milano-Bicocca, Veduggio al Lambro, Italy*

² *Pathology, Department of Medicine and Surgery, University of Milano-Bicocca, Fondazione IRCCS San Gerardo dei Tintori, Monza, Italy*

Keywords: MALDI-MSI, H&E WSI, co-registration, multi-modal, multi-omic

We propose an automatic co-registration method to align hematoxylin and eosin stained whole slide imaging data depicting tissue morphology with MALDI-MSI data depicting tissue molecular composition. The proposed approach unlocks the development of efficient multimodal computational pathology data integration pipelines to curate and annotate large-scale datasets. Matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI) enables interrogating the molecular composition of tissue with ever-increasing resolution and sensitivity. The output of a MALDI-MSI analysis is a sparse 3D tensor depicting the spatial distribution and relative ion intensity of molecular features. Gigapixel optical whole slide images (WSIs) can provide morphological characteristics of the same tissue section, due to the non-destructive nature of MALDI-MSI. The bimodal spatial analysis of the morpho-molecular signal might enhance downstream diagnostic tasks and offer crucial support to pathologists [1, 2]. We propose an operator-free and data-efficient co-registration toolbox using the ANTsX ecosystem [3]. We develop and validate an intensity-based method, inspired by the work of W.M. Abdelmoula [4], to estimate the global affine transform parameters linking the coordinate systems of the two spatially-resolved modalities: the MALDI-MSI (.imzML) and the H&E-stained WSI (e.g., .tif); additionally, we linked them to the SCiLS-Lab closed-source software (Bruker Daltonics, Germany). We tested the method on more than 50 image pairs from formalin-fixed paraffin-embedded (FFPE) tissue sections, comprising lung and thyroid tissue microarrays (TMAs), kidney and thyroid resections, and organoids. The molecular data is acquired with *timsTOFflex* and *rapifleX* instruments at a spatial resolution of 20 or 50 μm . Compared to a manual setting performed in SCiLS-Lab, in two thyroid TMAs of 86 patients, the proposed automatic method improved the co-registration, obtaining higher cell coverage (95 wins out of 132 cores) within histone-expressing regions and higher connective cells coverage (78 wins out of 118 cores) within collagen-expressing regions. Moreover, we propose a resampling method to align multiple omic levels acquired on the same tissue section, and consequently map them to the system of coordinates of the optical image. Overall, the proposed workflow enables efficient multimodal and multiomic spatial integration and paves the way for new translational studies.

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FACS-Proteomics strategy toward Extracellular Vesicles single-phenotype characterization in biological fluids: exploring the role of Leukocyte-derived EVs in Multiple Sclerosis

Maria Concetta Cufaro¹, Paola Lanuti¹, Domenico De Bellis¹, Serena Veschi², Anna Piro², Antonella Fontana², Alice Di Sebastiano¹, Davide Brocco¹, Pasquale Simeone¹, Serena Pilato², Reza M. H. Khorrooshi³, Valentina Tomassini⁴, Marianna Gabriella Rispoli⁴, Luca Federici¹, Ilaria Cicalini¹, Damiana Pieragostino¹, Piero Del Boccio¹

¹ Center for Advanced Studies and Technology (CAST), University "G. d'Annunzio" of Chieti-Pescara

² Department of Pharmacy, University "G. d'Annunzio" of Chieti-Pescara

³ Department of Neurobiology Research, Institute of Molecular Medicine, University of Southern Denmark, 5000 Odense, Denmark

⁴ Department of Neurosciences, Imaging, and Clinical Sciences, University "G. d'Annunzio" of Chieti-Pescara

Keywords: *Leukocyte-derived Extracellular Vesicles, Multiple Sclerosis, shotgun proteomics, Tears, EV Fluorescence Activated Cell Sorting isolation, nanoLC-MS/MS*

The isolation and proteomics characterization of Extracellular Vesicles (EVs) from body fluids is challenging due to their vast heterogeneity [1]. We have recently demonstrated that Fluorescence-activated Cell Sorting (FACS) efficiently isolates the whole EV circulating compartment directly from untouched body fluids enabling a comprehensive EV proteomics analysis [2]. In this study, we characterized, for the first time, a single-phenotype EV subset by sorting leucocyte-derived EVs (Leuko EVs) from peripheral blood and tears of healthy volunteers. Using an optimized and patented staining protocol of the whole EV compartment we identified and excluded non-EV particles, debris and damaged EVs. We further isolated, using an anti-CD45 antibody, Leuko EVs (CD45+ EVs), reaching a high level of purity (> 90%). Purified Leuko EVs were characterized using atomic force microscopy, nanoparticle tracking, and shotgun proteomics analysis revealing a similar coded protein cargo in both biological fluids. Subsequently, the same workflow was applied to tears from Relapsing-Remitting Multiple Sclerosis (RRMS) patients, revealing a Leuko EVs protein cargo enrichment that reflects the neuroinflammatory condition characteristics of RRMS. This enrichment was evidenced by the activation of upstream regulators TGFB1 and NFE2L2, which are associated with inflammatory responses. Additionally, the analysis identified markers indicative of endothelial cell proliferation and the development of enhanced vascular networks, with AGNPT2 and VEGF emerging as activated upstream regulators. These findings indicate the complex interplay between inflammation and angiogenesis in RRMS. In conclusion, our combined FACS-Proteomics strategy offers a promising approach for biomarker discovery, analysing cell-specific EV phenotypes directly from untouched body fluids, advancing the clinical value of tears EVs and improving the understanding of EV-mediated processes in vivo [3].

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Posters

Advanced 2D-HPLC–MS/MS Approach for Cosmetic Peptide Analysis in Skin Homogenates

Fosca Errante^{1,2}, Marco Pallecchi¹, Marta Menicatti¹, Lisa Giovannelli¹, Paolo Rovero^{1,2} and Gianluca Bartolucci¹

¹ Department of Neurofarba (Department of Neurosciences, Psychology, Drug Research and Child Health), University of Florence, Italy

² Interdepartmental Research Unit of Peptides and Proteins Chemistry and Biology, University of Florence, Italy

Keywords: Dermal stability, isomeric peptide analogues, scrambled peptide, Ion trap mass spectrometry, on-line sample cleanup

Peptides are increasingly employed as active ingredients in both therapeutic and cosmeceutical applications due to their high biological specificity, favorable safety profiles, and expanding market relevance [1]. In topical formulations, however, their efficacy is strongly influenced by stability within the dermal environment, where widespread proteolytic enzymes can promote degradation and limit bioavailability [2]. Despite the growing use of bioactive peptides in dermatology and cosmetics, robust analytical methodologies for assessing their resistance to enzymatic degradation in biologically relevant matrices remain limited. In this study, a two-dimensional (2D) HPLC–MS/MS method based on ion trap detection was developed and validated for the quantitative evaluation of peptide stability in human skin homogenate (HSH). The analytical platform integrates on-line sample clean-up with chromatographic separation on a silica-based pentafluorophenyl (PFP) column, enabling reliable analysis of peptides with markedly different polarity profiles within a single workflow. The method supports direct injection of samples containing a relatively high organic solvent content, allowing limited sample dilution, reducing sample handling, and preserving analytical sensitivity. The loading step was optimized to ensure efficient trapping of highly hydrophilic peptides, while minimizing matrix-related interferences. A key feature of the method is the use of isomeric peptide analogues (scrambled and reverse-sequence peptides) as internal standards. These compounds share identical precursor ions with their corresponding analytes and can be monitored under the same MS/MS conditions, ensuring effective correction for variability in ionization and fragmentation. This strategy represents a practical and scalable alternative to stable isotope-labelled standards, particularly in exploratory or screening studies. The ion trap mass analyzer further contributed to method robustness by providing controlled and reproducible fragmentation behavior with consistent product ion distributions. The validated method demonstrated satisfactory linearity, sensitivity, precision, accuracy, and minimal matrix effects. Its applicability was confirmed through stability studies of peptides with distinct polarity characteristics in HSH, highlighting different degradation behaviors under enzymatic conditions. Overall, the proposed 2D-HPLC–MS/MS approach provides a versatile analytical platform for peptide stability assessment in complex dermal matrices and supports early-stage screening and preclinical evaluation of bioactive peptides intended for topical applications.

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Systemic Exposure and Intratumoral Distribution of Paclitaxel after Nab-Paclitaxel administration via PIPAC in Pancreatic Adenocarcinoma with Peritoneal Metastases

Francesca Pagani^{1*}, Cristina Matteo¹, Marta Cancelliere¹, Amelia Locatelli², Davide Magrin², Alice Passoni², Fabio Pacelli³, Federica Ferracci³, Andrea Di Giorgio³, Massimo Zucchetti¹, Roberta Frapolli¹.

¹ Cancer Pharmacology Lab., Istituto di Ricerche Farmacologiche Mario Negri, Via M. Negri 2, Milan, 20156 (Italy)

² Mass Spectrometry for One Health Unit., Istituto di Ricerche Farmacologiche Mario Negri, Via M. Negri 2, Milan, 20156 (Italy)

³ UOC di Chirurgia del Peritoneo e Retroperitoneo, Largo A. Gemelli 8, Rome, 00168 (Italy)

Keywords: pancreatic adenocarcinoma, Nab-paclitaxel, PIPAC, UHPLC-MS/MS, MALDI

Pancreatic carcinoma is an aggressive malignancy characterized by a high metastatic potential and a 5-year survival rate of approximately 7%. Nabpaclitaxel combined with Gemcitabine represents the first-line systemic treatment for metastatic pancreatic adenocarcinoma, although survival benefits remain limited. Pressurized Intraperitoneal Aerosol Chemotherapy (PIPAC) is an innovative drug-delivery approach used to treat malignancies within the peritoneal cavity. Nab-paclitaxel administered through PIPAC (Nab-PIPAC), when combined with intravenous chemotherapy, may improve therapeutic efficacy in patients with peritoneal metastases. The aim of this study was to investigate systemic exposure and intratumoral penetration of paclitaxel (PTX) following Nab-PIPAC in patients with metastatic pancreatic adenocarcinoma enrolled in the Nab-PIPAC Trial (NCT05371223). The first three enrolled patients received two cycles of systemic Nab-paclitaxel (125 mg/m² i.v.), with the last dose administered 13 days before Nab-PIPAC (112.5 mg/m² nebulized intraperitoneally). Plasma samples, normal parietal peritoneum (NPP), and peritoneal metastasis (PM) biopsies were collected to quantify PTX levels and evaluate its tissue distribution. PTX concentrations in plasma and homogenized tissues were measured by UHPLC-MS/MS (Exion LC coupled with a 3500 triple quadrupole, AB SCIEX LLC). Spatial drug distribution in NPP and PM tissue sections was assessed using imaging mass spectrometry (IMS). A 1,5-diaminonaphthalene matrix containing PTX-d5 (3 µg/mL) as an internal standard was sprayed onto the MALDI plate. IMS analysis was performed using an Orbitrap Q-Exactive (Thermo Scientific) coupled with an atmospheric pressure MALDI source (MassTech). Plasma PTX levels were generally low and exceeded the neutropenia-associated toxic threshold (0.085 µg/mL) only at 2 and 4 hours after perfusion. In NPP and PM biopsies collected before Nab-PIPAC, mean PTX concentrations were consistently below 0.5 µg/g, likely reflecting residual drug accumulation from previous systemic administration. Notably, PTX concentrations in PM samples collected 30 minutes after Nab-PIPAC were higher than in NPP in two out of three patients. MALDI-MSI confirmed preferential PTX localization within metastatic nodules, whereas minimal signal was detected in adjacent normal peritoneum. These preliminary findings indicate that Nab-PIPAC achieves high local concentrations of PTX in peritoneal tissues while maintaining minimal systemic exposure.

Antigen Discovery in Membranous Nephropathy Using DIA-TIMS-PASEF Proteomics on Microdissected Glomeruli

Lisa Pagani¹, Viola Tintori¹, Clizia Chinello¹, Jan Ulrich Becker², Federico Alberici³, Martina Tedesco³, Vincenzo L'Imperio⁴ and Andrew Smith¹

¹ *University of Milano-Bicocca, Department of Medicine and Surgery, Proteomics and Metabolomics Unit, Veduggio al Lambro, Italy.*

² *University Hospital of Cologne, Institute of Pathology, Cologne, Germany.*

³ *ASST Spedali Civili di Brescia, Nephrology Unit, Spedali Civili Hospital, Brescia, Italy.*

⁴ *Fondazione IRCCS San Gerardo dei Tintori, Nephrology Unit, Monza, Italy*

Keywords: *Membranous Nephropathy, Laser Capture Microdissection, DIA-PASEF, Proteomics*

Membranous nephropathy (MN) is a glomerular disease characterised by subepithelial immune deposits leading to complement activation and progressive renal damage. Distinguishing primary from secondary forms is essential for clinical management, as treatment strategies differ significantly. Diagnosis integrates clinical and histopathological evaluation, with renal biopsy as the gold standard, supported by antigen identification (mainly PLA2R and THSD7A). Although new antigens have recently been described, current approaches remain limited by restricted biopsy material and the need for multiple antibody-based assays. Mass spectrometry-based proteomics offers a promising alternative for unbiased antigen identification [1,2].

In this study, we aimed to (i) develop a mass spectrometry method for antigen detection and assess its sensitivity, (ii) apply the method to a patient cohort to evaluate its performance, and (iii) perform a preliminary exploratory proteomics analysis comparing responders and non-responders to Rituximab therapy. Individual glomeruli were isolated by laser capture microdissection (LCM) and analysed by nano liquid chromatography–tandem mass spectrometry (nLC–MS/MS) in data-independent acquisition (DIA) mode, implemented with trapped ion mobility spectrometry coupled to parallel accumulation–serial fragmentation (TIMS-PASEF). Using this approach, PLA2R was detectable from as few as three glomeruli, with approximately 2700 protein identifications. Increasing the number of glomeruli improved protein coverage, reaching a plateau at ~25 glomeruli (~5000 protein IDs).

The method was applied to patient samples collected from multiple centres, analysing 10 glomeruli per case to determine the predominant antigen. Results were consistent with routine clinical findings, confirming PLA2R or THSD7A positivity where previously identified. Notably, in cases classified as double-negative, our approach enabled the identification of a putative MN-associated antigen in 65% of patients by comparison with reported antigen panels. The multiplexing capability of this technique allows comprehensive protein profiling without the need for additional tissue or specific antibodies. Furthermore, the same dataset enabled exploratory proteomic analysis, identifying ~70 differentially expressed proteins between responders and non-responders to Rituximab, with pathway analysis suggesting impaired glomerular filtration function and increased inflammatory activity in non-responders [4,5].

Overall, this approach demonstrates the potential of mass spectrometry to identify patient-specific MN antigens from minimal sample amounts, while simultaneously enabling in-depth proteomic investigation of disease mechanisms without additional tissue consumption. Moreover, it may serve as a valuable tool to support clinicians in diagnostically challenging cases.

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Exploring KDEL Receptor Oncogenic Signalling in Melanoma through Mass Spectrometry-Based Phosphoproteomics

Anna Giulia Ruggieri^{1,2}, Chiara Gramegna Tota^{1,2}, Marta Palumbo^{2,3}, Fabio Di Ferdinando^{1,2}, Federica Di Marco^{1,2}, Damiana Pieragostino^{1,2}, Vincenzo De Laurenzi^{1,2}, Luca Federici^{1,2} and Michele Sallese^{1,2}

¹ *Department of Innovative Technologies in Medicine and Dentistry, University “G. d’Annunzio” of Chieti-Pescara, Chieti, Italy*

² *Center for Advanced Studies and Technology (CAST) University “G. d’Annunzio” of Chieti-Pescara, Chieti, Italy*

³ *Department of Neuroscience, Imaging and Clinical Sciences, University “G. d’Annunzio” of Chieti-Pescara, Chieti, Italy*

Keynotes: *Phosphoproteomics, KDEL Receptors, Melanoma, Signalling networks*

Understanding how cancer cells reprogram their signalling networks to sustain growth and adaptability remains a major challenge in oncology. Addressing this complexity requires analytical approaches enable to map post-translational regulation at a systems level, and mass spectrometry-based phosphoproteomics, combining phosphopeptide enrichment with high-resolution LC–MS/MS, has proven particularly well suited to this task.

In this study we investigated the signalling role of KDEL receptors (KDEL1, KDEL2 and KDEL3) in a melanoma cell line.

KDEL receptors are seven-transmembrane proteins canonically involved in ER–Golgi trafficking and endoplasmic reticulum homeostasis. Beyond protein retrieval function, KDELs are increasingly recognised as signalling hubs that couple intracellular stress sensing to pathways involved in cell proliferation, autophagy and invasive behaviour. In particular, KDEL3 has been specifically implicated in metastatic dissemination in melanoma, which is a highly aggressive malignancy sustained by adaptable signalling circuits.

Analysis of large public datasets covering over 800 genome-wide molecular profiles revealed frequent KDEL alterations associated with poor clinical outcomes, while functional studies confirmed that KDEL silencing markedly impairs proliferation, motility and chemotaxis.

To explore the downstream signalling architecture, we used LC–MS/MS-based phosphoproteomics to profile phosphorylation events triggered by KDEL stimulation. Functional enrichment and network analysis revealed coordinated changes in the phosphorylation of proteins involved in cytoskeleton organisation, cell adhesion and vesicle-mediated trafficking.

Overall, these findings highlight the potential of MS-based phosphoproteomics to unravel non-canonical signalling mechanisms in cancer.

Multiplex biomarker quantification for the differential diagnosis of sphingolipidoses in lysosomal storage diseases

Gessica Di Carlo¹, Silvia Valentinuzzi¹, Mirco Zucchelli¹, Alberto Frisco¹, Maria Lucia Tommolini¹, Maria Concetta Cufaro¹, Luca Natale¹, Ilaria Cicalini¹, Ines Bucci¹, Damiana Pieragostino¹, Luca Federici¹, Vincenzo De Laurenzi¹, Claudia Rossi¹

¹ Center for Advanced Studies and Technology (CAST), University "G. d'Annunzio" of Chieti-Pescara, Chieti, Italy

Keywords: lysosomal storage diseases, LC–MS/MS, sphingolipidoses

Lysosomal storage diseases (LSDs) comprise a heterogeneous group of severe multisystem disorders caused by deficiencies in specific lysosomal enzymes, leading to the progressive accumulation of undegraded or partially degraded macromolecules within lysosomes. [1]

In the context of diagnosing LSDs, first-tier assays on dried blood spot samples evaluate enzymatic deficiencies, although the thermolabile nature of enzymes may cause activity loss, leading to false-positive results. Consequently, the implementation of a confirmatory test is necessary to improve diagnostic accuracy and to support in differential diagnosis among sphingolipidoses. The multiplex assay presented here evaluates the accumulation of specific biomarkers: glucosylsphingosine (LysoGb1) for Gaucher Disease (GD), galactosylsphingosine (Psychosine) for Krabbe Disease (KD), Lyso-Sphingomyelin (Lyso-SM) and Lyso-Sphingomyelin-509 (Lyso-SM-509) as markers of Acid Sphingomyelinase Deficiency (ASMD), and Globotriaosylsphingosine (LysoGb3) for Fabry Disease (FD).

Sphingolipid biomarker analysis was performed using liquid chromatography–tandem mass spectrometry (LC–MS/MS) in positive electrospray ionization mode (ESI+), following optimized extraction protocols applied to both standard solutions and biological matrices. Due to their diastereomeric relationship, Lyso-Gb1 and Psychosine share the same m/z value; therefore, conventional second-tier assays are generally unable to achieve their chromatographic separation. In the present method, this limitation was overcome through the implementation of hydrophilic interaction liquid chromatography (HILIC), which enabled effective chromatographic resolution of the two species. The total analytical run time was approximately 22 minutes per injection.

The clinical impact of this approach lies in its ability to separate Hexosylsphingosine species (LysoGb1 and Psychosine) that are currently detected as a combined signal in second-tier assays, providing an analytical tool to support the differential diagnosis between GD and KD. Incorporating Lyso-SM, Lyso-SM-509 and LysoGb3 biomarkers would broaden the spectrum of LSDs covered by the second-tier test.

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Proteomics characterization of pathological cranial sutures from mesenchymal calvarial stromal cells (CMCS) in scaphocephaly and trigonocephaly

Fabio Di Ferdinando¹, Simona Della Valle¹, Federica Di Marco¹, Piero Del Boccio¹, Damiana Pieragostino¹

¹ Center of Advanced Studies and Technology (CAST), University “G. d’Annunzio” of Chieti-Pescara, Chieti, Italy

Keywords: *craniosynostosis, CMCS, cranial sutures, proteomics characterization*

Craniosynostosis is characterized by the premature fusion of one or more cranial sutures, leading to abnormal skull growth. Among its most common forms, scaphocephaly and trigonocephaly arise from the early fusion of the sagittal and metopic sutures, respectively. Scaphocephaly is characterized by an elongated and narrow skull shape, while trigonocephaly presents a triangular forehead due to restricted frontal expansion. These morphological alterations may impair normal cranial growth and can be associated with altered brain development and potential neurocognitive deficits, including increased intracranial pressure and developmental delay [1]. In this study, we performed a label-free quantitative proteomic analysis on mesenchymal calvarial stromal cells (CMCS) isolated from fused (P) and non-fused (N) sutures in both scaphocephaly (SCA) and trigonocephaly (TRI) patients, to identify altered proteomic signatures in comparison: P SCA Vs N SCA and P TRI Vs N TRI. Protein samples were processed using the SP3-based tryptic digestion protocol and analyzed by nanoLC-Orbitrap-MS/MS [2]. A total of 928 proteins were robustly quantified using Proteome Discoverer (cut-off: p-value < 0.05 and |log₂ fold change| ≥ 1) and processed using Ingenuity Pathway Analysis (IPA) to perform functional enrichment analysis (|z-score| ≥ 2 and -log(p-value) ≥ 1.3). The pathological pictures in SCA and TRI reveal a stronger overall activation in scaphocephaly compared to trigonocephaly of canonical pathways mainly related to extracellular matrix organization and integrin-mediated signaling, as well as an increased binding of connective tissue cells and enhanced adhesion processes, supported by upstream molecular activation of TGF-β signaling. In contrast, trigonocephaly showed reduced activation of adhesion-related pathways, including integrin signaling, together with decreased binding of connective tissue cells and transmigration processes; at the upstream level, FGFR2 showed significant activation, whereas FGF8 exhibited complementary inhibition, suggesting an alteration in FGF-related signaling pathways. Notably, both conditions exhibited reduced cell viability and survival, indicating shared pathological features. Overall, quantitative proteomic analysis demonstrated that SCA and TRI display distinct expression profiles, clearly discriminating not only between pathological and control samples but also between the two conditions. In conclusion, while they share common altered pathways, they also exhibit divergent functional regulation, providing insight into the molecular pathogenic mechanisms in craniosynostosis and highlighting potential targets for future investigation and therapeutic strategies.

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DARTS-MS reveals Moesin and Galectin-3 as major targets of Silybin A in cancer cells

Emilio Brancaccio^{1,2}, Gilda D'Urso¹, Alessandra Capuano¹, Giovanni Di Fabio³, Valeria Romanucci³, Armando Zarrelli³, Francesco Pacifico⁴, Elvira Crescenzi⁴, Agostino Casapullo¹

¹Department of Pharmacy, University of Salerno, 84084 Fisciano, Italy.

²PhD program in Drug Discovery and Development, University of Salerno, 84084 Fisciano, Italy.

³Department of Chemical Sciences, University of Naples Federico II, Via Cintia 4, I-80126 Napoli, Italy.

⁴Istituto per l'Endocrinologia e l'Oncologia Sperimentale, Consiglio Nazionale delle Ricerche (CNR), Via S. Pansini, 5, 80131 Naples, Italy.

Keywords: Silybin A, limited proteolysis, DARTS, Moesin, Galectin-3.

Silymarin, the crude extract obtained from the fruits of *Silybum marianum* L. Gaertn., is mainly composed of flavonolignans (70–80%), a distinctive subclass of natural compounds consisting of a flavonoid unit linked to a phenylpropanoid moiety [1]. Among these, Silibinin (C₂₅H₂₂O₁₀), the diastomeric mixture of Silybin A and Silybin B, represents its principal and most abundant component [1]. Silybin A (2R,3R,10R,11R) and silybin B (2R,3R,10S,11S) are separated by preparative high-performance liquid chromatography (HPLC), enabling quick, gram-scale separation with purities higher than 98% [2]. Although stereochemical differences between silybin diastereomers result in distinct biological behaviors, the existing literature has predominantly focused on the interesting biological properties of the mixture [3], leaving the activities of the individual diastereoisomers insufficiently explored [1]. Therefore, this study focuses on the characterization of the Silybin A interactome within human cancer cells to identify its potential main protein targets, through application of Drug Affinity Responsive Target Stability (DARTS), a label-free MS-based functional proteomics technique based on limited proteolysis. DARTS is based on the principle that the interaction between a small molecule and a protein usually produces a structural thermodynamic stabilization of the target protein. Thus, the protein becomes more resistant to external perturbations, such as limited proteolysis, and this response can be detected and used to identify the proteins interacting with the small molecule, using data-dependent high-resolution mass spectrometry (DDA-MS) [4]. In the present study, DARTS experiments identified some putative protein targets, among which Moesin (MSN) and Galectin-3 (Gal-3) were selected as the most interesting, due to their crucial role in cancer physiopathology. Moesin is a member of the ezrin–radixin–moesin (ERM) family of proteins that link plasma membrane proteins to the cortical cytoskeleton, regulating different cellular processes. Moesin has a crucial role in epithelial morphogenesis, adhesion, and migration, three key events in the induction of a cancer phenotype [5]. Galectin-3 is a β -galactoside-binding lectin, frequently upregulated in several cancers, that contributes to tumor progression by promoting cell proliferation, resistance to apoptosis and invasion [6]. DARTS findings were validated with in vitro limited proteolysis on lysates coming from cells treated with Silybin A, through Western Blotting analysis. Moreover, cell migration assays suggested that Silybin A affects cell migration, potentially through modulation of Moesin and Galectin activity.

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A unified approach for renal amyloid detection and typing through mass spectrometry imaging and computational pathology

Greta Bindi¹, Sara Ghirardi¹, Vasco Coelho¹, Giorgio Cazzaniga², Fabio Pagni², Vincenzo L'imperio², Andrew Smith¹

¹*Department of Medicine and Surgery, Proteomics and Metabolomics Unit, University of Milano-Bicocca, Veduggio al Lambro, Italy*

²*Department of Anatomy and Pathology, IRCCS San Gerardo dei Tintori, Monza, Italy, Monza, Italy*

Keywords: MALDI-MSI; Renal pathology; MALDI-HI-PLEX-IHC, Multi-modal Imaging

Introduction

The kidney is one of the most common organs involved in systemic amyloidosis, frequently leading to renal failure and nephrotic syndrome. Moreover, management of renal amyloidosis typically involves therapy targeting the underlying amyloid process and, thus, the accurate detection and typing of the amyloid deposits are of paramount importance in order to define the appropriate therapeutic strategy. In this context, the simultaneous detection of the so-called 'universal amyloid signature' and the amyloid precursor proteins with MALDI-MSI has shown promise as a possible supportive tool, more so given its potential to be integrated with digital and computational pathology approaches.

Methods

A combined MALDI-MSI and computational pathology workflow was applied to a retrospective cohort of amyloid-positive renal biopsies sourced from the pathological archives of multiple clinical centres. Briefly, amyloid detection was achieved using our previously established MALDI-MSI workflow [1] and an automated computational pipeline that considers percentile-based intensity thresholds and intersecting pixels that display the presence of the 'amyloid signature'. Moreover, on the same bioptic tissue section, diagnostically relevant antibody probes targeting the most frequently occurring precursor proteins were conjugated with photocleavable mass tags (AmberGen, Inc.) to perform MALDI-based immunohistochemistry (MALDI-IHC) for amyloid typing.

Main results

Following optimisation of the computational pipeline, it was possible to detect renal amyloid deposits that showed a coherent spatial correlation with those indicated by Congo Red (CR) fluorescence [2]. Moreover, from within the annotated amyloid deposits, it was possible to determine the amyloid precursor protein based upon the relative intensity of the associated PC-MT, even in those instances that presented as specific IHC positivity and this underlines the value of a framework that permits amyloid detection and typing on a single tissue section.

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Direct MALDI-MSI on H&E-stained sections enables multimodal spatial tissue analysis and computational pathology integration

Natalia Shelly Porto¹, Vasco Coelho¹, Vincenzo L'Imperio^{2,3}, Fulvio Magni¹, Vanna Denti¹

¹ Proteomics and Metabolomics Unit, Department of Medicine and Surgery, University of Milano-Bicocca, Milano, Italy

² Pathology Unit, Department of Medicine and Surgery, University of Milano-Bicocca, Milano, Italy

³ I.R.C.C.S San Gerardo, Monza, Italy

Keywords: Mass Spectrometry Imaging, Computational Pathology, Lung Cancer

Mass spectrometry imaging (MSI), particularly matrix-assisted laser desorption ionization (MALDI-MSI), enables in situ molecular analysis of archival tissues, supporting translational and clinical applications. Its non-destructive ionization allows subsequent histological staining [1]. However, post-MALDI-MSI tissue quality often falls short of standard haematoxylin and eosin (H&E) whole-slide imaging due to chemical processing and laser-induced artifacts, limiting true multimodal integration. Here, we propose a workflow that combines untargeted MALDI-MSI with H&E staining on the same section, enabling quantitative assessment of tissue morphology using pathology foundation models for cell segmentation (e.g., CellViT [2]) alongside spatial molecular data.

Two consecutive FFPE sections from a lung cancer tissue microarray were analysed: one using a conventional MALDI-MSI workflow with post-acquisition H&E staining, and the other was stained with H&E prior to MSI. In the latter case, following staining and whole-slide image (WSI) acquisition, the coverslip and the mounting medium were removed by toluene washes before re-entering the standard MALDI-MSI preparation protocol for tryptic peptides. MSI data were acquired in positive ion mode on a timsTOF fleX instrument, operating at 20 µm/px lateral resolution. WSIs were digitised using a NanoZoomer S60 at a magnification of 40x. Cell populations were quantified using a visual transformer, CellViT [2] across replicate cores, stained post- or pre- MSI respectively.

Pre-staining with H&E prior to MALDI-MSI did not adversely affect peptide signal detection, yielding comparable molecular coverage relative to the standard workflow. Importantly, no evidence of analyte delocalisation was observed, with spatial distributions of detected peptides remaining consistent with histological features and comparable to the conventional protocol. This confirms that the spatial integrity of molecular signals is preserved despite prior staining. In addition, this approach significantly improved WSI quality, enabling more accurate and robust cell segmentation and classification, leading to more cells being detected and correctly classified.

These results show that H&E staining prior to MALDI-MSI is compatible with 20 µm/px spatial proteomics, preserving molecular signal intensity and spatial localisation while improving downstream computational pathology analyses. This optimized workflow enhances multimodal data integration, supporting quantitative spatial biology and advancing the use of MALDI-MSI in translational and clinical research.

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Mass Spectrometry-based proteomic and intact glycopeptide analysis of colorectal cancer tissue

Simona Liuzzi¹, Caterina Gabriele¹, Davide La Padula², Vittoria Rago³, Michele Ammendola¹, Silvia Di Agostino², Vittorio Abbonante², Marco Gaspari¹

¹ Department of Experimental and Clinical Medicine, Magna Graecia University, Catanzaro, Italy

² Department of Health Sciences, Magna Graecia University, Catanzaro, Italy

³ Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Rende, Italy

Keywords: Glycoproteomics, CRC, Tissue

Aberrant glycosylation is a key feature of colorectal cancer (CRC), influencing tumor cell behavior, cell–matrix interactions and the tumor microenvironment. While proteomics captures changes in protein abundance, intact glycopeptide analysis provides an additional level of information by linking each glycan structure to its specific peptide and protein context [1]. In this study, we combined total proteomics and intact glycopeptide analysis of matched tumor and non-tumor tissues from 31 CRC patients.

For glycopeptide analysis, 20 µg of protein extracts were digested using a protein aggregation capture protocol, purified by strong cation exchange, enriched by cotton-based HILIC [2] and analyzed by high-resolution LC-MS/MS on an Orbitrap Exploris 240 in data-dependent acquisition mode. Glycopeptide identification was performed using Byonic within Proteome Discoverer 2.5. In parallel, 10 µg of protein extracts were processed using the same digestion and purification workflow and analyzed by high-resolution LC-MS/MS on an Orbitrap Exploris 480 in data-independent acquisition mode for total proteomics. Proteomic data were processed using Spectronaut v.18.7, and statistical analysis was performed using Perseus v2.0.11.0.

The glycopeptide analysis identified 5178 intact glycopeptides assigned to 543 unique proteins. Among them, 2303 glycopeptides were significantly altered in tumor tissues, including 745 upregulated and 1558 downregulated features. Glycan-class analysis revealed distinct patterns between upregulated and downregulated glycopeptides, with high mannose-class structures enriched among upregulated features and fucosylated/sialylated-fucosylated classes more represented among downregulated ones. Total proteomics identified 6930 proteins, of which 472 overlapped with proteins assigned from glycopeptide analysis, while 71 were detected only at the glycopeptide level. Shared proteins highlighted CRC-relevant processes, including cell adhesion, extracellular matrix/mucosal remodeling and inflammation/immune response. CEACAM5, FCGBP and MUC2 showed extensive glycoform heterogeneity, whereas COL1A1 and COL3A1 displayed partially discordant protein- and glycopeptide-level trends. Among glycopeptide-only proteins, CHST11 showed 15 significantly upregulated glycopeptides, suggesting glycosaminoglycan-related extracellular matrix remodeling not captured by total proteomics. Overall, this integrated MS-based approach shows that intact glycopeptide analysis adds a complementary molecular layer to proteomics, revealing glycan-class redistribution, protein-specific glycoform heterogeneity and tumor-associated features that would otherwise remain partially unresolved.

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Integrated DIA and Thermal Proteome Profiling of Lamotrigine-Treated MCF-7 Cells

Annarita Giuliano¹, Elena Ricci², Caterina Gabriele¹, Mariarosa Fava², Sofia Spadafora², Catia Morelli², Diego Sisci², Marco Gaspari¹

¹*Research Centre for Advanced Biochemistry and Molecular Biology, Department of Experimental and Clinical Medicine, Magna Græcia University of Catanzaro, 88100 Catanzaro, Italy*

²*Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, 87036 Rende, Italy*

Keywords: lamotrigine, data-independent acquisition (DIA), thermal proteome profiling (TPP)

Lamotrigine, a clinically approved anticonvulsant, has recently emerged as a promising candidate for drug repurposing in breast cancer [1]. However, the molecular mechanisms underlying its potential antitumor activity remain poorly understood. In this study, an integrated proteomic approach combining data-independent acquisition (DIA) and thermal proteome profiling (TPP) [2] was employed to elucidate the cellular effects and potential targets of lamotrigine in MCF-7 breast cancer cells.

DIA analysis quantified 6,622 proteins, including 142 significantly regulated upon lamotrigine exposure. Functional enrichment analysis identified mitochondrial energy metabolism, oxidative phosphorylation, chromatin organization, and protein translation as the main affected processes. TPP analysis showed significant thermal stability alterations in ABRAXAS2, MT-CYB, TMEM97, and MTA2.

Of note, mitochondrial alterations were observed in both analyses, with MT-CYB being downregulated by DIA and thermally destabilized by TPP, supporting mitochondrial dysfunction as a potential central mechanism of lamotrigine action. Western blotting confirmed reduced MT-CYB expression, while reactive oxygen species (ROS) production indicated increased oxidative stress upon lamotrigine treatment.

Overall, this combined strategy enabled the simultaneous assessment of protein abundance changes and thermal stability shifts, providing complementary insights into drug-induced proteomic alterations. These results provide a mechanistic basis for further investigation of lamotrigine as a repurposed anticancer agent in breast cancer.

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Metabolic signatures of resistance to Sorafenib and Immune Checkpoint Inhibitors (ICIs) in Hepatocellular Carcinoma

Gabriele Imperato¹, Silvia Pedretti¹, Miriana Braghin¹, Francesca Palermo¹, Meral Celikag¹, Anna Maltoni², Marta Boccazzi¹, Veronica Vallelonga², Francesco Gandolfi², Ilario Giovanni Rapposelli³, Michele Zanoni³, Nicola Zamboni⁴, Serena Ghisletti^{1,2}, Maurizio Crestani¹, Emma De Fabiani¹ and Nico Mitro^{1,2}

¹Dipartimento di Scienze Farmacologiche Biomolecolari, Università degli Studi di Milano, 20133 Milano, Italy

²Dipartimento di Oncologia Sperimentale, Istituto Europeo di Oncologia (IEO), 20139 Milano, Italy

³Dipartimento di Oncologia Medica, IRCCS Istituto Romagnolo per lo Studio dei Tumori, 47014 Meldola, Italy

⁴Institute of Molecular System Biology - Eidgenössische Technische Hochschule (ETH), 8093 Zürich, Swiss

Keywords: cancer, metabolism, Sorafenib, Immune Checkpoint Inhibitors, metabolomics analysis

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and is associated with high mortality worldwide. Treatment options depend on disease stage and include surgical resection, liver transplantation, or local ablative approaches for early-stage disease, whereas systemic therapies are used for advanced or unresectable HCC. These include multikinase inhibitors, such as sorafenib, and immune checkpoint inhibitors (ICIs), including atezolizumab plus bevacizumab, durvalumab, and tremelimumab. However, both therapeutic strategies can be limited by the development of drug resistance, ultimately compromising treatment efficacy. In this project, we investigated two distinct therapeutic settings in HCC. In the first study, we used targeted metabolomics and untargeted lipidomics to dissect the metabolic adaptations underlying sorafenib response and resistance, and to identify circulating biomarkers of drug activity. In the second, ongoing study, we explored metabolic alterations associated with response to ICIs in patient plasma samples.

In the sorafenib study, we demonstrated that sorafenib impairs mitochondrial oxidative metabolism in HCC cells, promoting metabolic reprogramming toward glycolysis and lipid remodeling. In sensitive cells, increased glycolytic flux leads to the accumulation of toxic methylglyoxal, which is detoxified through the glyoxalase pathway to produce D-lactate. D-lactate accumulation is associated with sorafenib activity and contributes to cell death through ferroptosis-related mechanisms. In contrast, resistant cells escape this vulnerability through metabolic rewiring involving the glycerol-3-phosphate pathway, which supports NAD⁺ regeneration, glycerolipid metabolism, and membrane remodeling, thereby protecting cells from ferroptotic damage. These mechanistic findings led to the identification and validation of circulating biomarkers in plasma from HCC patients treated with sorafenib: increased D-lactate was associated with treatment response, whereas elevated glycerol was linked to resistance and disease progression.

In a separate, ongoing study focused on ICI-treated HCC patients, preliminary targeted metabolomics analyses of plasma samples indicate that the kynurenine/tryptophan pathway may be involved in treatment response. In responding patients, kynurenine levels increased from baseline, possibly reflecting IFN γ -driven activation of the indoleamine 2,3-dioxygenase pathway following T-cell reactivation. This suggests that kynurenine may represent a potential marker of an active antitumor immune response. In contrast, non-responders showed a lower kynurenine/tryptophan ratio and reduced tryptophan levels compared with baseline.

In conclusion, our sorafenib study identifies D-lactate and glycerol as promising circulating biomarkers for monitoring sorafenib activity in HCC, with D-lactate associated with response and glycerol with resistance. In parallel, our ongoing investigation of ICI-treated patients highlights the kynurenine/tryptophan pathway as a potentially relevant metabolic axis associated with immunotherapy response. Further studies are required to validate these preliminary findings and to assess their clinical utility as predictive or pharmacodynamic biomarkers in HCC.

Mass spectrometry-based analysis of serum metabolome in malnutrition and sarcopenia: preliminary results from SENIOR study

Maura Brioschi¹, Stefania Pagliari¹, Matteo Emilio Federico Capietti¹, Nagaia Madini², Sara Santero², Hellas Cena^{2,3}, Flavia Magri^{4,5}, Luca Campone¹

¹ Department of Biotechnology and Biosciences, Università degli Studi di Milano Bicocca, Milan, Italy

² Laboratory of Dietetics and Clinical Nutrition, Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Pavia, Italy.

³ Istituti Clinici Scientifici Maugeri IRCCS, Unit of Clinical Nutrition, Pavia, Italy

⁴ Department of Internal Medicine and Therapeutics, University of Pavia, Pavia, Italy

⁵ Unit of Endocrinology and Metabolism, Laboratory for Endocrine Disruptors, Istituti Clinici Scientifici Maugeri IRCCS, Pavia, Italy

Keywords: Sarcopenia, malnutrition, metabolomics

Malnutrition is one of the most pressing problems globally. In Italy, the most affected are low-income families, people with diseases, and elderly individuals. Additionally, the group of people at risk of malnutrition is growing due to the ageing population and the consequential increase in the burden of diseases and health care costs. Sarcopenia is a progressive and generalized skeletal muscle disorder associated with an increased likelihood of adverse outcomes including falls, fractures, physical disability, and mortality. Sarcopenia is linked to several risk factors, including malnutrition, immobilization, disease, and inflammation [1].

The SENIOR project is a cross-sectional multicentre study aimed to assess the prevalence of malnutrition and sarcopenia and the impact of lifestyle, health status and quality of life on the occurrence of these conditions in patients over 65 years of age. This study aims to identify new circulating biomarkers of sarcopenia and malnutrition, representing new research products based on already collected data.

To address this issue a mass spectrometry-based metabolomic approach have been performed to discover novel circulating biomarkers of sarcopenia severity and malnutrition. After solvent extraction, serum metabolites have been separated by liquid chromatography on a UPLC HClass coupled to a Xevo G2-XS qTOF mass spectrometer (Waters Corporation) through an ESI source. Progenesis QI (Waters) and Metaboanalyst software were used for data processing and statistical analysis.

Comparing the metabolomic profile of 38 patients with overt and severe sarcopenia and 37 non-sarcopenic subjects, multivariate analysis allowed to identify a total of 39 discriminating features with Q value < 0.05 and a VIP value > 1 in PLS-DA analysis. In conclusion, these preliminary results suggest that metabolomic profile analysis might be regarded as a possible tool to discover predictive biomarkers of sarcopenia progression. Discriminating metabolites resulting from multivariate analysis will be also analysed in correlation with data on lifestyle and inflammatory profile.

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Development of multi-omics approach for biomarker and risk factor identification

C. D. Calvano^{1,2}, V. Nettis¹, A. Castellaneta¹, G. Ventura^{1,2}, M. Bianco^{1,2}, I. Losito, T.R.I. Cataldi^{1,2}, S. S. Tangaro³

*¹Dipartimento di Chimica, ²Centro SMART, ³Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti
Università degli Studi di Bari Aldo Moro, 70126 Bari, Italy*

Keywords: *omics, big data, sample extraction, integrated MS platform*

In this project we will employ a multi-omics analysis where high-resolution mass spectrometry platforms (Orbitrap, MALDI, LC-MS/MS) will be used to acquire lipidomic, metabolomic, and proteomic data from tissues, plasma, urine, and liquid biopsies. Predictive models where machine learning algorithms will be employed to identify diagnostic patterns from integrated multi-omics datasets will be the key point of improved digital technologies for early biomarker discovery. The first experimental phase includes acquiring training samples (plasma) to refine the preparation protocol in terms of reproducibility, number of extracted analytes, simplicity, and minimal handling. From collected plasma, metabolites, lipids, and proteins will be isolated following established protocols including the use of single organic solvent extraction (SOSE), two-phase liquid-liquid extraction (LLE) assisted by ultrasound (UAE) such as the Folch method or the Bligh-Dyer protocol for lipids with UAE, acetone precipitation or ultracentrifugation for protein isolation, and extraction in medium polarity solvents like ethanol to water for metabolites. Variations in the ratios of chloroform-methanol-water in LLE, use of additives, or alternative solvents like MTBE and butanol or more effective detergents such as sodium deoxycholate have been tested. The effectiveness of each extraction procedure will be tested using HILIC or RPLC separation coupled to ESI-MS/MS analysis, selecting the strategy that yields the greatest amount of information. Specifically, lipid mixtures will be analyzed with hydrophilic interaction liquid chromatography (HILIC) for lipid class separation, while metabolites and peptides will be analyzed with reversed-phase chromatography [1,2]. The use of complementary MS techniques (linear ion trap or high-resolution Orbitrap) can provide a comprehensive view of the omics fractions in relatively short times. The selected protocols will be standardized on replicates of the same sample to ensure omics data reproducibility. After selecting the appropriate protocol, the exploratory phase of untargeted multi-omics analysis will begin. In the next steps, this integrated study of the metabolome, lipidome, and proteome on biological matrices will be conducted on control and diseased samples to preliminarily identify features associated with pathological conditions using the support of digital technologies.

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Inside the Cup: Exploring Coffee Aroma through Explainable Artificial Intelligence

Giorgio Felizzato¹, Luciano Navarini², Cecilia Cagliero¹, Andrea Caratti¹, Giorgia Botta¹, Chiara Cordero¹, Erica Liberto¹

¹Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, Via Pietro Giuria 9, 10125 Torino, Italy

²Illycaffè S.p.A., Via Flavia 110, 34147 Trieste, Italy

Keywords: *specialty coffee; origin identity; SHAP; aroma intensity; explainable AI*

Coffee quality assessment is a complex task influenced by multiple factors, including geographical origin, processing conditions, and roasting degree, all of which shape the volatile composition and sensory perception of the final beverage. In particular, aroma intensity represents a key attribute for both consumer perception and industrial product classification, yet its relationship with chemical composition remains only partially understood.

In this work, headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME/GC–MS) was employed to characterise the volatile profiles a set of roasted and ground single-origin coffee samples from five different provenances. The analytical workflow enabled the detection and semi-quantitative analysis of a wide range of volatile organic compounds, providing a comprehensive chemical profile of the samples that includes pyrazines, furans, pyrroles, terpenes, and phenolic derivatives.

To extract meaningful chemical information from the GC–MS data, machine learning models and explainable AI methods were applied. A Support Vector Machine classifier achieved 91% accuracy in discriminating coffee origins based on GC–MS data, demonstrating its strong potential for origin authentication. Model interpretability was enhanced using SHapley Additive exPlanations (SHAP), which allowed the identification of key compounds driving classification, highlighting origin-specific chemical markers related to roasting-derived reactions and precursor composition.

In addition, a Ridge Regression model was developed to predict aroma intensity values (scale 1–9) reported on product packaging. The model showed high predictive performance ($R^2 = 0.88$; RMSE = 0.61), establishing a quantitative relationship between GC–MS volatile profiles and perceived sensory intensity. Key contributors to perceived intensity were identified among pyridines, pyrazines, lactones, furans, and pyrroles, supporting the role of roasting chemistry in defining sensory strength.

Overall, this study demonstrates how GC–MS-based volatilomics, combined with explainable artificial intelligence, can provide both predictive accuracy and chemical interpretability. The proposed approach offers a framework for coffee quality control, enabling the linkage between molecular composition, geographical origin, and sensory attributes. This strategy represents a step toward more objective, data-driven quality assessment systems in the coffee industry, bridging analytical chemistry and sensory science.

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Proteomic Characterization of Processed Hazelnuts: Implications for Hypoallergenic Food Development

Elena C. L. Rigante¹, Laura Quintieri¹, Anna Luparelli¹, Federica De Bellis¹, Linda Monaci¹

¹ *Institute of Biomembranes, Bioenergetics and Molecular Biotechnologies, National Research Council (IBIOM-CNR), Bari, Italy*

Keywords: *nuts allergy, thermal treatment, hypoallergenic foods, bottom up-proteomics, in silico allergens prediction*

Hazelnut allergy represents one of the most common food allergies, often associated with severe IgE-mediated reactions [1]. Advanced techniques such as proteomics and genomics allowed to identify several hazelnut proteins responsible for triggering allergic reactions in sensitive individuals. These proteins tend to be highly stable and resistant to digestion, which increases their potential to provoke an immune response [2]. Additionally, the allergenic properties of hazelnut proteins can be influenced by food processing methods. Certain treatments - such as hydrolysis, fermentation, and heating - have been explored and optimized to modify or reduce their allergenicity [3-5]. This study investigates how specialized processing - moving beyond simple thermal treatment - can modulate the hazelnut proteome for hypoallergenic applications. The experimental design focused on the synergistic effect of moisture and pressure: roasted hazelnut kernels and derivative powders were first hydrated in water and subsequently subjected to high-pressure saturated steam in an autoclave at 134 °C for 10 minutes. This thermo-hydrometric treatment aims to induce deep structural modifications in allergenic proteins. Protein extracts from native and processed samples were analyzed using a bottom-up proteomic approach via UHPLC-ESI (+)-ddHRMS, complemented by targeted Selected Ion Monitoring (SIM) to track specific peptides. Identified proteins were subjected to in silico allergenicity assessment by querying the Immune Epitope Database (IEDB). Particular attention was devoted to major hazelnut allergens, such as Cor a 8 (Lipid Transfer Protein) and Cor a 9 (11S Globulin), specifically targeting peptide sequences associated with known IgE-binding epitopes. The results revealed significant alterations in protein profiles following processing, with evidence of reduced detection of specific allergenic proteins and marked modifications in peptide patterns. Epitope mapping suggested a substantial decrease in sequences associated with allergenic potential indicating that the applied treatments effectively compromise the structural integrity and potential immunoreactivity of hazelnut proteins. The investigation highlighted that the high-pressure saturated steam treatment (autoclave), particularly when preceded by hydration (HA samples), was significantly more effective in reducing the presence of most allergenic epitopes compared to dry-treated (A samples) or untreated controls. Notably, the sample matrix influenced the response: hazelnut powders showed a more pronounced and variable reduction in specific sequences compared to whole roasted kernels. Autoclaving appears promising for producing hypoallergenic ingredients, but residual allergenic peptides persist, requiring further optimization. The study supports mass spectrometry-based proteomics for allergenicity assessment, with future work including in vitro digestion and clinical validation to confirm safety.

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Mass spectrometry-based characterization of bacteriocins from *Leuconostoc mesenteroides* MS4 active against *Xylella fastidiosa*

Mariachiara Bianco^{1,2}, Cosima Damiana Calvano^{1,2}, Martina Solarino¹, Miloud Sabri³, Toufic Elbeaino³, Tommaso Cataldi^{1,2}

¹ Dipartimento di Chimica, Università degli Studi di Bari Aldo Moro, 70126 Bari, Italy

² Centro Interdipartimentale SMART, Università degli Studi di Bari Aldo Moro, via E. Orabona 4, 70126 Bari, Italy

³ International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM of Bari), Via Ceglie 9, Valenzano, Bari 70010, Italy

Keywords: proteomics, top-down, polypeptides, antimicrobial activity

Xylella fastidiosa subsp. *pauca* (Xfp) represents a major threat to Mediterranean agriculture, and current control strategies remain largely ineffective, highlighting the urgent need for novel and sustainable antibacterial agents. In this study, we investigated the antimicrobial potential of *Leuconostoc mesenteroides* strain MS4 against Xfp through a combination of *in vitro* and *in planta* approaches. Cell-free precipitate (CFP) obtained from MS4 cultures inhibited Xfp growth on BCYE agar, whereas proteinase K treatment abolished this activity, indicating the proteinaceous nature of the active compounds. Electron and fluorescence microscopy revealed extensive membrane disruption and cellular damage in treated Xfp cells, consistent with a bactericidal mechanism. Whole-genome sequencing identified a circular chromosome (1860,891 bp) and a plasmid (37,317 bp), including six putative bacteriocin-encoding genes (45–59 amino acids). Targeted proteomic analyses were carried out using complementary mass spectrometry approaches. MALDI-TOF/TOF MS (Matrix-assisted laser desorption ionization-time of flight--mass spectrometry) spectra of untreated samples displayed low-intensity signals in the 4000–6500 *m/z* range, consistent with the presence of high-molecular-weight (HMW) polypeptides, which disappeared after proteinase K digestion, confirming their proteic nature. Conversely, the 1000–3000 *m/z* region became enriched in lower-mass peptides following enzymatic digestion. Peptide mass fingerprinting (PMF) using proteinase K enabled the identification of several proteins associated with *Leuconostoc*, although none could be directly correlated with the experimentally observed at HMW. To overcome this limitation, a top-down strategy was applied using reversed-phase liquid chromatography (RPLC)-electrospray (ESI)-high resolution (HR)MS. The analysis revealed multiple-charge-state distributions, whose deconvolution allowed the accurate mass determination of at least four peptides. Ions at *m/z* 819.798, 983.757, and 1229.446 were assigned to peptide MK-45 with charge states of +6, +5, and +4, respectively, while the ion at *m/z* 1186.430⁵⁺ corresponded to peptide MG-58. Similarly, ions at *m/z* 917.017⁶⁺, 1100.218⁵⁺, and 1375.023⁴⁺ were attributed to peptide MR-53, and ions at *m/z* 877.333⁷⁺, 1023.386⁶⁺, 1227.864⁵⁺, and 1534.577⁴⁺ to peptide MW-56. Structural characterization was further supported by RPLC-ESI(+)-MS/MS experiments performed on both low- and high-resolution instruments, combining collision-induced dissociation (CID) and higher-energy collisional dissociation (HCD) to improve sequence coverage. These analyses confirmed the production of four bacteriocins (MK-45, MR-53, MW-56, and MG-58) in the cell-free supernatant [1]. Overall, MS4 represents a promising and cost-effective source of bacteriocins, and MS proved essential for their identification and structural characterization, supporting the development of sustainable strategies against *Xylella*-associated diseases.

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HarmoNICo – Proteomics Interlaboratory Testing for the BBMRI.it-NICo Network

Maria Concetta Cufaro¹, Romina Belli², Daniele Peroni², Fiorenza Farè³, Emma De Fabiani³, Marialuisa Casella⁴, Serena Camerini⁴, Beatrice Muscatello⁵, Marinella De Leo⁵, Clizia Chinello⁶, Andrew James Smith⁶, Marcello Manfredi⁷, Daniela Sorio⁸, Francesca Griggio⁸, Valentina Adami², Piero Del Boccio¹

¹ Center for Advanced Studies and Technology (CAST), University “G. d’Annunzio” of Chieti-Pescara, 66100, Chieti, Italy

² Cellular, Computational and Integrative Biology (CIBIO), University of Trento, 38112, Trento, Italy

³ University Technological Platforms (UNITECH-OMICS), University of Milan, 20139, Milan, Italy

⁴ Core Facilities Technical-Scientific Service (FAST), Istituto Superiore di Sanità, 00161, Rome, Italy

⁵ Center for Instrument Sharing (CISUP), University of Pisa, 56126, Pisa, Italy

⁶ Mass Spectrometry Platform, University of Milano-Bicocca, 20126, Milan, Italy

⁷ Center for Translational Research on Autoimmune and Allergic Disease (CAAD), University of Eastern Piedmont, 28100 Novara, Italy

⁸ Center for Technological Platforms (CPT), University of Verona, 37134 Verona, Italy

Keywords: mass spectrometry-based proteomics, DIA, DDA, interlaboratory test, NICo

In the era of big data, reproducibility in proteomics relies on protocol harmonization and adherence to FAIR principles, especially within distributed research infrastructures [1]. To address this challenge, the HarmoNICo initiative and the NICo (Network of Italian Core facilities) - BBMRI.it Mass Spectrometry Working Group performed a pilot interlaboratory study aimed at assessing the performance, comparability, and variability of proteomics workflows across core facilities.

Eight laboratories analyzed a standardized, commercially available, HeLa protein digest as a complex reference sample. Each facility performed label-free LC-MS/MS analyses using in-house nanoLC-MS/MS platforms, applying both Data-Dependent Acquisition (DDA) and Data-Independent Acquisition (DIA) strategies, with five analytical replicates per sample. While flexibility in instrumentation, chromatographic setups, and acquisition parameters was allowed to reflect real operating conditions, sample input and preparation were standardized. To reduce variability at the computational level, DDA datasets from participating laboratories were centrally processed using a harmonized bioinformatics pipeline (Proteome Discoverer v2.2.0), including database searching (MASCOT v2.6.2) with controlled false discovery rate (FDR < 1%), log2 transformation, median normalization, missing value imputation, and batch effect correction.

The results showed high inter-laboratory reproducibility, with approximately 77% overlap in quantified proteins and consistent protein abundance profiles across sites. Principal Component Analysis initially revealed laboratory-specific clustering, which was largely mitigated after normalization and batch correction.

Overall, this study demonstrates the robustness of proteomics workflows across the NICo network and highlights the critical role of standardized data processing in ensuring reproducibility. These findings represent a significant step toward methodological harmonization, ultimately enhancing quality, comparability, and translational relevance, facilitating the development of proteomics best practices of the network.

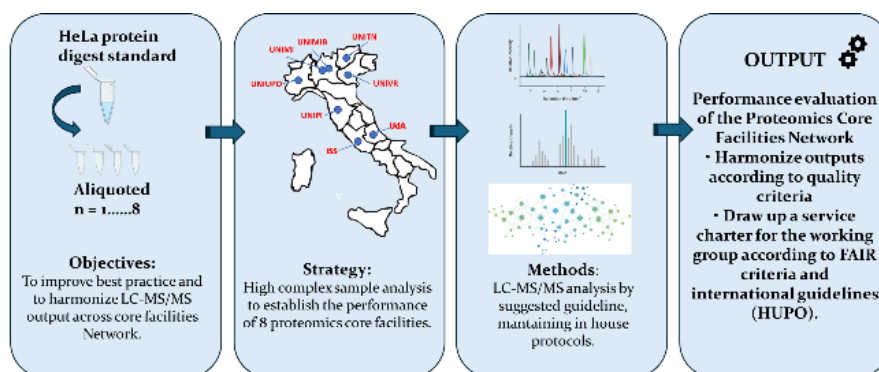


Figure 1. Experimental workflow showing the laboratories involved in the pilot study, the strategies adopted and the objectives set.

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High-resolution mass spectrometry for metabolomic profiling of agri-food by-products: a comparison of extraction strategies

Orsolina Vetrano^{1,2}, Maria Assunta Crescenzi¹, Daniele Sanna³, Angela Fadda⁴, Paola Montoro¹

¹ Department of Pharmacy, University of the Study of Salerno, Via Giovanni Paolo II 132, 84084 Fisciano, Italy

² PhD Program in Drug Discovery & Development, Pharmacy Department, University of the Study of Salerno.

³ Institute of Biomolecular Chemistry, National Research Council, Traversa La Crucca 3, 07100 Sassari, Italy

⁴ Institute of the Sciences of Food Production, National Research Council, Traversa la Crucca 3, 07100 Sassari, Italy

Keywords: circular economy, food waste valorization, phenolic compounds, green extraction (NaDES), UHPLC-HRMS

The valorization of food waste has emerged as a key strategy within the framework of the circular economy. In this context, the progression toward more sustainable agro-industrial systems demands innovative approaches to effectively exploit underutilized resources. The recovery and valorization of food by-products therefore represent a powerful opportunity to reduce waste while simultaneously enhancing food quality and promoting sustainable resource management [1].

Raw materials such as olive (*Olea europaea* L.) leaves, strawberry (*Fragaria* × *ananassa*) leaves, and myrtle (*Myrtus communis* L.) liqueur by-products were selected due to their abundance, underutilization, and high content of bioactive compounds. Olive leaves represent about 5% of the biomass processed during oil production and are typically discarded or burned, strawberry leaves are mostly residual from large-scale cultivation in Campania, and myrtle by-products amount to approximately 150,000 kg per year in Sardinia but are currently used only as soil improvers or combustible material [1].

The present study explores and compares advanced extraction strategies to maximize phenolic recovery. Non-conventional techniques, including ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE), are evaluated alongside innovative green extraction systems based on Natural Deep Eutectic Solvents (NaDES) [2,3]. Particular emphasis has been placed on assessing the extraction efficiency and selectivity of NaDES as sustainable alternatives to traditional solvents. Particular attention is devoted to the development of post-extraction purification methods, especially for extracts obtained using NADES [3].

A comprehensive metabolomic approach is employed to achieve chemical characterization of the extracts. Phenolic profiling is carried out using ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-Q Exactive™ Orbitrap-MS/MS) which allows high accuracy mass measurements and the reliable identification of both major and minor compounds. Metabolite annotation was performed using a workflow combining Compound Discoverer software with public spectral libraries and use of standard compounds. Finally, Multivariate Data Analysis was applied to compare extracts [4]. By integrating green extraction technologies with high-resolution mass spectrometry and Multivariate data analysis, this work aims to unlock the full potential of by-products as a source of functional ingredients, thereby contributing to waste reduction, resource efficiency, and innovation in the food sector [1,4].

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Comparative Phenolic Profiling of *Malus domestica* (cv. Golden vs. GoldRush) Agricultural By-products: An LC-MS Approach for the Valorisation of Green Pruning and Thinning Residues

Mathieu Merlet¹, Tania Flutto¹, Simona Leveque¹, Sabina Valentini¹

¹ *Institut Agricole Régional, 11100 Aosta, Italy*

Keywords: LC-ESI-MS, natural plant protection, circular economy, bioactive compounds

Apple (*Malus domestica*) cultivation generates significant biomass through green pruning and fruit thinning. While typically discarded, these residues are rich in bioactive phenolics with potential health and agricultural applications [1,2]. In disease-resistant cultivars like GoldRush, resistance to apple scab (*Venturia inaequalis*) is often linked to an enhanced synthesis of these secondary metabolites [3]. This study compares the phenolic profiles of residues from the resistant GoldRush and the susceptible Golden Delicious to identify strategies for valorising these agricultural by-products.

Thinning and pruning residues collected in June (Aosta, Italy) were processed via Accelerated Solvent Extraction (ASE) [4] using water/ethanol mixtures. Extracts were screened for Total Polyphenolic Content (TPC) and Antioxidant Activity (AOA). Detailed profiling was performed via LC-ESI-MS, with data processed through MS-DIAL and metabolite identification supported by accurate mass, fragmentation patterns, and external standards.

The analysis revealed significantly higher TPC values in all GoldRush samples compared to Golden Delicious (mean values of 4563.81 mg/L and 2602.37 mg/L, respectively). These findings were corroborated by AOA assays, where GoldRush reached 60.35 mg TE/g, more than double the activity of Golden Delicious (24.89 mg TE/g). Regarding the detailed phenolic profile, GoldRush exhibited a superior accumulation of chlorogenic acid, procyanidin B2, epicatechin, and flavonols such as hyperoside, quercetin 3-glucoside, and kaempferol 3-glucoside. Interestingly, GoldRush showed a lower concentration of phloridzin compared to Golden Delicious.

The superior antioxidant potential and phenolic complexity of GoldRush residues highlight the dual benefit of resistant varieties: reduced pesticide reliance and the production of high-value bioactive biomass. These waste materials are ideal candidates for nutraceuticals and natural plant protection products. Future research will evaluate these specific metabolic markers as biopesticides against *V. inaequalis* to support a circular economy in alpine fruit growing.

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UHPLC/QTOF Investigation of Changes in Anthocyanin Structures in Indigenous Sicilian Rosé Sparkling Wines Produced by the Charmat-Martinotti Method

Annarita Panighel¹, Mirko De Rosso¹, Antonio Sparacio², Salvatore Sparla^{2,3}, Luciano Vettori⁴,
Riccardo Velasco¹, Francesca Tarricone¹, Riccardo Flamini¹

¹ Council for Agricultural Research and Economics - Viticulture & Oenology (CREA-VE), Viale XVIII aprile 26, 31015 Conegliano (TV), Italy

² Istituto Regionale del Vino e dell'Olio - Regione Sicilia (IRVO), Via Libertà 66, 90100 Palermo, Italy

³ Interdipartimental Center for Research in Viticulture and Enology (CIRVE) – Padua University, viale XXVIII Aprile 14, 31015 Conegliano (TV), Italy

⁴ Labor Wine Srls, 31058 Susegana (TV), Italy

Keywords: UHPLC/QTOF, anthocyanin, Sicilian rosé wine, sparkling, Charmat-Martinotti

According to EU Regulation n. 1308/2013/EC, "sparkling wine" is defined as a product resulting from the first or second fermentation of fresh grape, juice, or base wine, with a minimum alcoholic content of 8.5% v/v. This product must release fermentative carbon dioxide at a minimum pressure of 3 bar at 20 °C when the container is opened [1]. The second fermentation process can occur either through the addition of base wine and a yeast and sugar solution into the bottle - requiring several months and known as the Traditional Method - or through use of pressurized stainless steel tanks (autoclaves), which facilitate fermentation concluding in 1 to 2 weeks, referred to as the Charmat-Martinotti Method. France, Italy, Spain, Germany, and Russia are identified as the primary producers of sparkling wines, yielding an overall production of approximately 1.5 billion bottles, which constitutes over 70% of global production [2]. Currently, around 80 wineries in Sicily are engaged in the production of sparkling wines, with about 25% of this output comprising rosé wines. Following the significant success of Prosecco sparkling wines, there has been a notable interest in the production of rosé sparkling wines utilizing the Charmat-Martinotti method. Frappato, Nero d'Avola, and Perricone are local Sicilian red grape varieties traditionally associated with still wine production. In this study, sparkling wines from these grape varieties were experimentally produced using 28 L autoclaves. Anthocyanins are crucial to the overall quality of red and rosé wines, and the color is influenced by both grape variety and vinification processes used. While the impact of fermentation on anthocyanin composition of rosé wines have been investigated [3], the effects of the Charmat-Martinotti fermentation process on their composition in rosé sparkling wines have yet to be examined. This research focuses on the changes in anthocyanin composition of Frappato, Nero d'Avola, and Perricone rosé wines, by characterizing their structures using UHPLC/QTOF before and after refermentation via the Charmat-Martinotti method.

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Upcycling Agri-Food By-Products for Functional Snacking: Metabolomic and Sensory Insights into Fortified Traditional Italian Taralli

Leonardo Caputo¹, Federica De Bellis¹, Anna Luparelli¹, Laura Quintieri¹, Linda Monaci¹

¹ National Research Council of Italy, Institute of Biomembranes, Bioenergetics and Molecular Biotechnologies (CNR-IBIOM), Via Amendola G. 122/O, 70126 Bari, Italy.

Keywords: LC-HRMS/MS, Metabolome mapping, Tritordeum, Lentil hulls, Flavonoids

Tarallo Pugliese (TAP) is a cornerstone of the Southern Italian baking tradition and recognized as a Traditional Agri-Food Product (PAT). Despite its strong cultural roots, its functional and sustainable evolution represents a critical challenge for the modern food industry.

The proposed innovation lies in the fortification of the tarallo through the targeted integration of lentil hulls—a fiber-rich de-hulling by-product—and Tritordeum™, a natural cereal hybrid between wild barley and durum wheat valued for its excellent protein and antioxidant profile. The research focused on creating balanced blends capable of significantly enhancing the snack's nutritional profile without disrupting its traditional artisanal production process. A distinguishing element of this investigation was the focus on the impact of traditional "double-cooking" method - boiling followed by baking - on the stability of bioactive compounds without modifying the characteristic texture of this product.

The multidisciplinary analytical approach integrated descriptive sensory evaluations with high-resolution mass spectrometry (LC-HRMS/MS) techniques. Advanced data analysis allowed for an exploration of how the synergy between these new raw ingredients can compensate for the potential organoleptic limitations often associated with fiber addition, while simultaneously enhancing the product's aromatic complexity. Metabolome mapping revealed a significant retention of flavonoids and protective molecules, demonstrating the food matrix's ability to preserve nutrients during the double thermal treatments.

In summary, this study demonstrates that it is possible to transform a traditional snack into a high-value-added functional food. The results highlight how the integration of sustainable ingredients ensures stability, sensory acceptability, and potential wellness benefits, promoting a circular economy model that is practically applicable on an industrial scale. Moreover, this study shows a cutting-edge strategy based on the upcycling of agri-food by-products and the use of resilient crops, fully aligned with the United Nations Sustainable Development Goals (SDG 3 and 12) for responsible consumption and health.

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Inorganic mass spectrometry at Gran Sasso National Laboratories: a general overview

Francesco Ferella¹

¹ *Laboratori Nazionali del Gran Sasso, Istituto Nazionale di Fisica Nucleare, 67100 Assergi (L'Aquila), Italy*

Keywords: *astroparticle physics, low background, mass spectrometry*

Astroparticle physics experiments must be conducted in environments with extremely low contamination to achieve the sensitivity required for detecting rare events. Underground laboratories provide ideal conditions in this respect. The Gran Sasso National Laboratory (LNGS), the largest underground research facility in the world, is shielded by a thick rock overburden that reduces the cosmic ray flux by a factor of one million. In addition, the neutron flux is suppressed by roughly three orders of magnitude compared to surface levels, owing to the very low concentrations of thorium and uranium in the surrounding rock.

LNGS hosts a wide range of astroparticle physics experiments, particularly those focused on neutrino physics and dark matter searches, while also supporting other research areas such as underground biology. The primary objective of these experiments is to continuously improve sensitivity, which strongly depends on achieving ultra-low background conditions. A major source of background arises from the intrinsic radioactivity of the materials used in the experimental apparatus.

For this reason, the selection and use of highly radiopure materials are essential. Comprehensive screening campaigns are carried out to evaluate impurity levels and to verify that specific materials or production batches meet the stringent requirements for low-background applications. Among the techniques employed for this purpose, mass spectrometry plays a key role. In our facility, it is possible to analyze a wide variety of materials, enabling the identification and selection of the most suitable ones for different applications.

MS as a Supporting Tool for UHPLC-DAD Method Development and Validation: The Case of 16-O-Methylcafestol in Coffee

Luciano Navarini¹, Elena Guercia²

¹ *Illycaffè SpA, Trieste, Italy*

² *Aromalab, Area Science Park, illycaffè SpA, Trieste*

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Mass spectrometry is commonly employed for both targeted and untargeted analyses; however, it can also play a crucial role in supporting and validating new analytical methods developed using other techniques such as UHPLC with different detectors. This is particularly relevant for the analysis of coffee diterpenes, and especially for the quantification of 16-O-methylcafestol (16OMC).

16OMC has long been considered a molecular marker for differentiating *Coffea canephora* (Robusta) from *C. arabica* (Arabica). In recent years, however, trace amounts of 16OMC have been detected in Arabica coffees [1], prompting the development, optimization and validation of increasingly sensitive analytical methods capable of its reliable quantification at low concentration levels [2]. From a chemical characterization perspective, there is also strong interest in the simultaneous quantification of other coffee diterpenes, namely cafestol, kahweol, dehydrocafestol and dehydrokahweol for authenticity purposes.

In the present work, a standardized official method (DIN method 10779, 2011) was improved by replicating, on a UHPLC system, the analytical conditions previously described by Guercia et al. [2] for LC–MS analysis. This approach allowed not only the quantitative determination of the major diterpenes by UHPLC-DAD, but also a qualitative determination of 16OMC (presence/absence) as a preliminary screening step, to be subsequently complemented by quantitative LC–MS analysis.

Quantitative results obtained by UHPLC-DAD and LC–MS were compared using both interlaboratory proficiency test samples and green coffee samples (*C. stenophylla*) [3]. The support of mass spectrometry proved to be essential for confirming analyte identity and for establishing a robust preliminary screening method, enhancing confidence in the overall analytical workflow for coffee diterpene characterization.

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Antibiotic-based molecular networking development for advanced screening in food samples

Marinella Tano¹, Fabiola Eugelio¹, Sara Palmieri¹, Federico Fanti¹, Giampiero Scortichini², Michele Del Carlo¹, Dario Compagnone¹

¹ *Dipartimento di Bioscienze e Tecnologie Agro-Alimentari ed Ambientali, Università degli Studi di Teramo, 64100 Teramo, Italy;*

² *Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", 64100 Teramo, Italy*

Keywords: *Molecular networking; Antibiotic residues; Food safety*

The presence of antibiotic residues in food products has become a growing concern worldwide due to its implications for public health, food safety, and the emergence of antimicrobial resistance. [1] Antibiotics are widely used in veterinary medicine and livestock production to prevent and treat infections, but their improper use or insufficient withdrawal periods can lead to residual compounds persisting in food products such as milk, meat, and derived goods. Continuous exposure of antibiotics through diet may contribute to the development of resistant bacterial strains, posing a serious threat to global health systems. For this reason, reliable, sensitive, and comprehensive screening methods are essential to ensure regulatory compliance and protect consumers. [2]

In this context, this presentation focuses on the development of an antibiotic-based molecular networking strategy for advanced screening in food samples. The proposed approach combines high-resolution mass spectrometry (HRMS) with computational molecular networking tools to enable both targeted and untargeted analysis of antibiotic residues. Unlike conventional screening methods, which are often limited to predefined compounds, molecular networking leverages spectral similarity to group structurally related molecules, allowing the detection of known antibiotics as well as their metabolites, degradation products, and potentially unknown contaminants. [3]

The developed workflow aims to enhance analytical performance by improving sensitivity, selectivity, and data interpretation in complex food matrices, such as meat, milk, and eggs. Through the visualization of molecular relationships, it becomes possible to identify clusters of compounds and reveal hidden chemical patterns that would otherwise remain undetected. Preliminary applications on representative food samples demonstrate the capability of this approach to differentiate closely related antibiotic structures and to expand the screening coverage beyond traditional methods.

Overall, this strategy represents a promising advancement for food safety monitoring, offering a more holistic and efficient tool for the detection of antibiotic residues and supporting efforts to mitigate the risks associated with antimicrobial resistance.

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Beyond Traces: Advanced HRMS Diagnostics for the Identification of Micropollutants in Soil

Monica Brienza¹, Gianluigi Buttiglieri², Rocco Iunnissi³, Filomena Lelario¹, Hana Mazoz⁴, Laura Scrano^{1,5}, Sabino Aurelio Bufo^{1,6}, Achille Palma³

¹ Department of Basic and Applied Sciences (DiSBA), University of Basilicata, 85100, Potenza, Italy

² Catalan Institute for Water Research (ICRA-CERCA), 17003 Girona, Spain

³ Agenzia Regionale per la Protezione Ambientale, 85100, Potenza, Italy

⁴ Claude Bernard University, 69100, Lyon, France

⁵ IRSA, 74100, Taranto, Italy

⁶ Johannesburg University, 2006, South Africa

Keywords: micropollutants, soil, HPLC-HRMS, advanced diagnostic

Soil contamination by synthetic organic compounds represents a critical environmental challenge. Unlike aquatic ecosystems, soil serves as a reservoir where contaminants can persist or transform into complex metabolites. The complexity of the matrix and trace concentrations often render conventional analytical methods inadequate. This study presents an advanced diagnostic approach based on high-performance liquid chromatography coupled to high-resolution mass spectrometry (HPLC-HRMS), applied to soil samples from the Basilicata region.

The samples were dried, homogenized, and subjected to ultrasound-assisted extraction. The extracts, purified by SPE, were analyzed with an Accelera 600 HPLC system coupled to a Q-Orbitrap HRMS spectrometer equipped with a HESI probe. The analysis revealed the presence of fipronil-desulfinyl at concentrations consistent with local water monitoring. However, the HRMS approach also identified carbamazepine and salicylic acid, substances not detected in water, demonstrating the soil's ability to sequester contaminants that escape water monitoring.

In conclusion, the high molecular precision of the Q-Orbitrap system has overcome the limitations of traditional methods, ensuring a more accurate, sensitive, and representative environmental risk assessment of the true chemical pressure on terrestrial ecosystems.

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The Role and Importance of Eurachem in Supporting Quality and Accreditation in Analytical Laboratories

Marina Patriarca¹, Emanuela Gregori²

¹ Eurachem Italia, già ISS, viale Regina Elena, 299 00161 Rome, Italy

² Eurachem Italia, Dip. Sicurezza Alimentare, Nutrizione e Sanità Pubblica Veterinaria, ISS, viale Regina Elena, 299 00161 Rome, Italy

Keywords: method validation, uncertainty

In recent years, Eurachem has further strengthened its role through the publication of new technical guides and major updates that are highly important for accredited laboratories. These documents provide practical support for implementing the requirements of the ISO/IEC 17025 standard and for improving quality management in analytical activities. Among the most significant recent publications is the fourth edition of Guide to Quality in Analytical Chemistry: An Aid to Accreditation (2026), updated to reflect the requirements introduced by ISO/IEC 17025:2017. The guide provides operational recommendations on quality management, analytical process control, method validation, metrological traceability, and staff technical competence.

Eurachem has also released updated guidance on analytical method validation, including The Fitness for Purpose of Analytical Methods (2025) and related supplements concerning validation study design and the management of analytical blanks. These documents help laboratories demonstrate the reliability and suitability of analytical methods while reducing systematic measurement errors. Particularly important is the new guide The Fitness for Intended Use of Analytical Equipment and Systems (2025), which focuses on the qualification and lifecycle management of analytical instruments and systems. The guide addresses modern laboratory challenges such as risk-based approaches, data integrity, quality principles, instrument performance monitoring, and compliance with GMP and GLP requirements.

Another key area recently addressed by Eurachem is measurement uncertainty. The guide Evaluation of Measurement Uncertainty from In-house Precision and Recovery Data (2026) supports laboratories in estimating uncertainty using internal precision and recovery data in a way that is both realistic and compliant with accreditation and regulatory expectations. Through these updated guides, international workshops, and specialist working groups, Eurachem continues to be an essential reference for chemical, microbiological, and clinical laboratories. Its activities promote the harmonization of analytical procedures, continuous quality improvement, and international comparability of analytical results, thereby increasing confidence in laboratory data across scientific, industrial, environmental, pharmaceutical, and regulatory sectors.

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