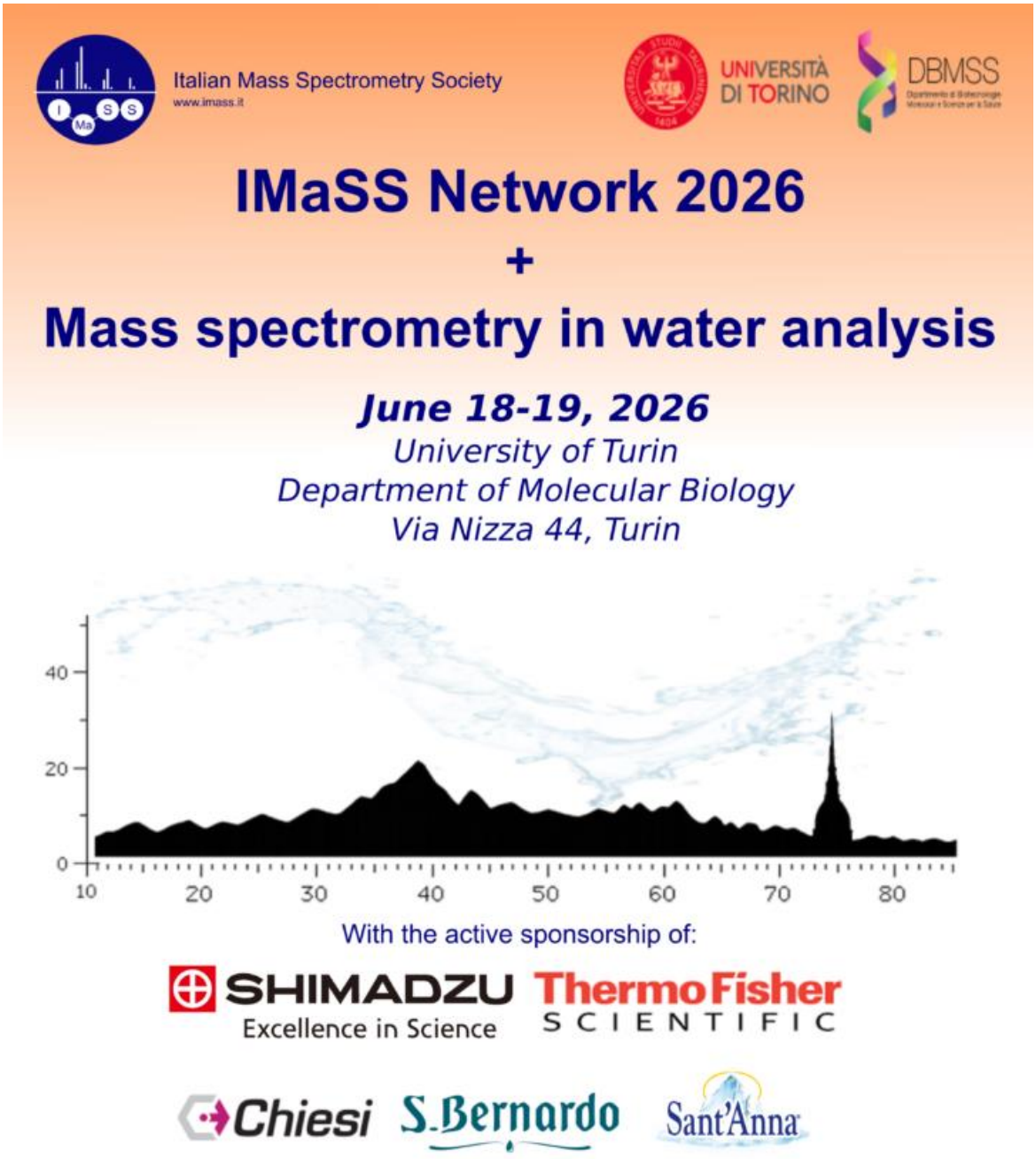
# Book of abstracts



## Abstract index

# ORAL CONTRIBUTIONS

# FROM ALPINE SNOW TO MEDITERRANEAN RIVERS: NON-TARGET ANALYSIS OF CECS IN CONTRASTING AQUATIC ENVIRONMENTS

Mónika Adányi[1,2], Manuel A. Andino-Enriquez[3], Masho Hilawie Belay[1], Alessia Fabbris[1], Arianna Ghignone[1], I. Vallés[2], A. Arques[2],Paola Calza[3], Emilio Marengo[1], Elisa Robotti[1]

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Contaminants of emerging concern (CECs) are increasingly detected in aquatic environments, including remote areas. Non-target analysis based on high-resolution mass spectrometry is a powerful strategy for characterizing complex mixtures, identifying chemical fingerprints, and prioritizing compounds not routinely monitored. This presentation compares two contrasting case studies: surface snow and river waters from the Monte Bianco area in the Italian Alps, and surface waters from the Serpis River in Spain. In the Monte Bianco study, an integrated approach combining LC-HRMS, GC-MS, and complementary extraction workflows revealed 284 identified contaminants, including industrial additives, plasticizers, pharmaceuticals, flame retardants, pesticides, ultraviolet filters, and tire-derived compounds. Multivariate chemometric analysis highlighted altitude-dependent and hydrological gradients along the snow–water continuum. The results indicate that high-mountain environments can act as reservoirs of legacy and contemporary-use chemicals, with remobilization during snowmelt. In the Serpis River study, water samples from 17 sites were analyzed by solid-phase extraction followed by non-target UPLC-HRMS using an Orbitrap Exploris 120 mass spectrometer *(Fig. 1)*. A total of 329 compounds were annotated at confidence Level 2, mainly pharmaceuticals, endogenous metabolites, industrial chemicals, pesticides, and personal care products. Multivariate analysis revealed spatial patterns associated with wastewater treatment plant discharges, urban inputs, and industrial activities. Together, these studies demonstrate the value of combining non-target HRMS with multivariate analysis to investigate complex aquatic systems.

**Fig. 1:** Orbitrap Exploris 120 mass spectrometer

**Acknowledgements:**

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# TFA AND ULTRA-SHORT CHAIN PFAS IN WATERS: A TARGETED HPLC-MS/MS SCREENING

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Ultra-short chain PFAS (USC-PFAS) such as trifluoroacetic acid (TFA), represent an emerging class of persistent organic contaminants of growing environmental concern. TFA is highly water-soluble, chemically stable, and increasingly detected in aquatic environments worldwide, primarily through atmospheric degradation of hydrofluorocarbons (HFCs) and hydrofluoroolefins (HFOs). Despite its ubiquitous occurrence, TFA and other USC-PFAS remain largely unregulated and systematically undermonitored in most water matrices.

This study presents the first systematic screening of TFA and seven other USC-PFAS (PFPrA, PFBA, TFMSA, PFEtSA, PFPrSA, PFBSA) in a broad set of water samples from Italy and Asia, using a validated high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) method. The proposed method showed excellent linearity ($R^2 \geq 0.993$), good accuracy (BIAS% < ±15%) and precision (RSD% < 10%), with LOQ values as low as 0.10–0.15 µg/L for most analytes. More than 130 water samples (tap, bottled, spring and surface waters) from different countries and world areas have been analyzed. Among all eight target analytes, only TFA was detected, confirming its unique environmental behavior and dominant role among USC-PFAS. Mineral waters showed the lowest average (0.30 µg/L), followed by tap waters (0.38 µg/L) and well waters (0.48 µg/L). Surface waters displayed the highest variability, with a mean of 0.63 µg/L. Notably, the highest TFA level recorded (2.02 µg/L) was found in a rainwater sample, highlighting the significant contribution of atmospheric deposition to water contamination.

International comparison revealed distinct geographical patterns. Thai waters (five bottled mineral and three tap waters) showed no detectable TFA in any sample, consistent with the widespread use of reverse osmosis purification in Thailand [1]. In contrast, Chinese samples (one bottled, one tap, one surface water) all contained measurable TFA concentrations (0.56, 0.85, and 1.85 µg/L, respectively), higher than average Italian values and consistent with previously reported atmospheric accumulation in urbanized Chinese regions [2].

These findings confirm TFA as a persistent and ubiquitous contaminant in European and Asian water resources and demonstrate that water treatment technology plays a critical role in determining final contamination levels.

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# GREEN UHPLC-MS/MS METHODS FOR THE MONITORING OF EMERGING POLLUTANTS IN COMPLIANCE WITH EU DIRECTIVES

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Emerging contaminants pose a risk to the environment due to their persistence and strong biological activity, leading to a notable pollutant potential to aquatic ecosystems [1]. In response, both the European Union (EU) and single European countries have recently issued updated regulatory frameworks for the monitoring of these pollutants. This study presents the development and validation of two green methods for the detection and quantification, by UHPLC-MS/MS, of two classes of pollutants in water matrices, both compliant with EU legislation:

- 29 compounds from the EU watch list (Directive 2025/439), including pharmaceuticals, cosmetic ingredients, fungicides and industrial chemicals, monitored in wastewater.
- 55 PFAS, including 30 as defined by Italian D.Lgs. 102/2025, monitored in drinking water.

A key aim was to achieve the sub-ppt limits of quantification (LOQs) required by the EU directives through direct injection, eliminating the need for any preconcentration step or other treatment procedures with significant environmental impact. This objective was met for all target compounds, including the most challenging ones (e.g., avermectin B1a, etoxazole and fipronil). All 55 PFAS were quantifiable at 0.5 ng/L, fully complying with current law. Both methods were optimised and validated, with each allowing determination of its target analytes in a 14-minute chromatographic run. Calibration was performed over the 5-800 ng/L range for EU watch list compounds and the 0.5-600 ng/L range for PFAS, with $r^2 > 0.9934$ for all analytes. Method precision, expressed as RSD at three concentration levels, was always below 13%, and recovery was in the range 86.7% - 105.6%.

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# DUAL-SPE UNTARGETED LC-ORBITRAP HRMS PROFILING OF EMERGING CONTAMINANTS IN THE CHAO PHRAYA RIVER

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The Chao Phraya River is Bangkok's main water artery and receives substantial agricultural and urban effluents across its basin, yet systematic untargeted monitoring for emerging contaminants remains scarce. Surface water was collected at two contrasting sites, an upstream agricultural reach (DO 8.1 mg/L) and a hypoxic downstream estuary (DO 1.96 mg/L; $Cl^-$ 60 mg/L), and extracted by dual solid-phase extraction (Oasis HLB + Envi-Carb) prior to LC-Orbitrap HRMS analysis (full scan/ddMS², ESI±). Feature extraction was performed in MZmine 4.9 and structural annotation with SIRIUS 6/CSI:FingerID (Schymanski levels 2a–3 [1]). The two sorbents showed complementary chemical-space coverage, recovering 311 and 240 sorbent-exclusive features, respectively (ESI+). Among 76 exact spectral library matches, a clear spatial gradient emerged (Figure 1): pharmaceuticals (carbamazepine, valsartan, fluconazole, cetirizine) and industrial compounds (TBEP, nonylphenol metabolites) were markedly enriched at the estuarine site, while pesticides (bispyribac, bentazon, ametryn) and LAS surfactant biodegradation products dominated upstream.

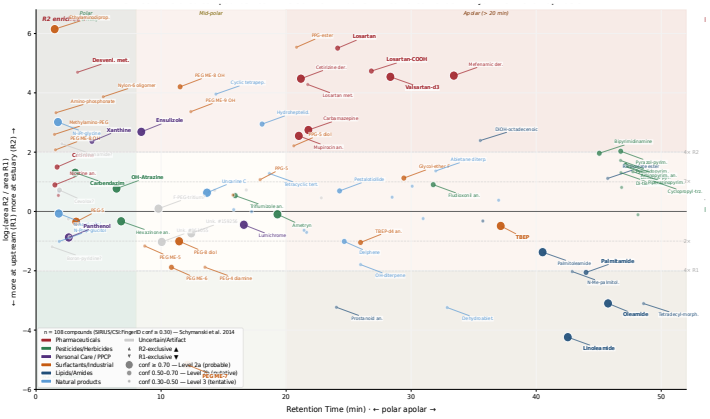


**Fig. 1:** RT vs $\log_2$(estuary/inlet) for the annotated features (conf ≥ 0.30), colored by chemical class.

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**Acknowledgements:**

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# APPLICATIONS OF A MINIATURE DEPLOYABLE MASS SPECTROMETER WITH SFC/APCI AND A COLD PLASMA ION SOURCE

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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 both with an SFC preparative chromatograph and with a ring-shaped cold plasma ion source (SICRIT, Plasmion, Augsburg). 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.

A Sepiatech SFC-250 multisample system (Sepiatech GmbH, Germany), equipped with a DAD detector, was coupled to the (MTE-30, MassTech Inc., USA) using a HILIC column. The mobile phase was carbon dioxide (A) and methanol (B), with a gradient from 10% to 50% B. Four isolated alkaloids were identified from Chelidonium majus (Greater Celandine), a pharmaceutically important species of the Papaveraceae family, as chelidonine, protopine, allocryptopine, and coptisine. Because two of these compounds, chelidonine and protopine, share the same molecular weight, MS/MS analysis was performed to confirm their structural identities

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.Here we 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.

# BEYOND LC-MS/MS: COLORIMETRIC AND FLUORIMETRIC SENSING STRATEGIES FOR PFAS AND FLUORIDE DETECTION IN WATER

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Per- and polyfluoroalkyl substances (PFAS) are persistent, bioaccumulative contaminants associated with endocrine disruption, immunotoxicity, and increased cancer risk. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) remains the reference technique for PFAS quantification, offering the sensitivity, selectivity, and validated performance required by the recast EU Drinking Water Directive 2020/2184 (0.1 µg/L for the sum of 20 priority PFAS, in force since January 2026) [1]. Its instrumental complexity, cost, and limited portability, however, constrain decentralized, high-throughput, and field-based screening, motivating the development of complementary analytical tools.

This contribution critically reviews recent advances in colorimetric and fluorimetric sensors as rapid, low-cost techniques for PFAS and fluoride detection in water. Several recognition mechanisms are discussed: localized surface plasmon resonance shifts induced by analyte-driven aggregation of gold and silver nanoparticles; oxidase-like activity of fluorinated metal–organic frameworks (e.g., F-Ce-UiO-66-NH2); ion-pair formation with cationic dyes (methylene blue assay); supramolecular host–guest chemistry (guanidinocalix[5]arenes); amplifying fluorescent polymers; and luminescence quenching of quantum dots and carbon dots [2]. Cross-reactive sensor arrays coupled with chemometric (PCA, LDA) and machine-learning tools overcome the structural similarity of PFAS congeners, enabling multiplexed discrimination at nanomolar levels. Fluoride-ion quantification, via lanthanum-chelate colorimetric assays or RNA-based fluorogenic biosensors (e.g., FluorMango), provides a stoichiometric proxy for C–F bond cleavage in defluorination studies, offering orthogonal information for monitoring chemical, electrochemical, and enzymatic remediation processes [3].

Most optical sensors still operate above current regulatory thresholds, remain susceptible to interferences from natural organic matter, surfactants, and competing ions, and cannot quantify oxidizable precursors or total organofluorine (AOF/EOF). They should therefore be regarded not as replacements for LC-MS/MS, but as a complementary tier within tiered monitoring frameworks, suitable for rapid screening, source identification, and remediation verification, pending systematic interlaboratory validation against certified reference materials.

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**Acknowledgements:**

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# MICROPLASTICS ANALYSIS IN WATER: REGULATORY REQUIREMENTS, STANDARDISATION ACTIVITIES AND FUTURE PERSPECTIVES

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Microplastics (MPs), generally defined as synthetic polymer particles smaller than 5 mm, are now recognized as ubiquitous contaminants in aquatic environments and have been detected in drinking water, wastewater, food products and human biological samples [1]. Their widespread occurrence has led to increasing regulatory attention, including the recent restriction of intentionally added MPs under the European REACH regulation and the ongoing activities of the EFSA, which has been requested by the European Commission to evaluate exposure and potential human health risks associated with micro- and nanoplastics in food [2]. However, significant data and methodological gaps still limit robust exposure and risk assessment. Water represents one of the main pathways for the transport and potential human exposure to MPs. In Europe, a major regulatory milestone was the inclusion of MPs within the Drinking Water Directive (EU) 2020/2184, followed by Commission Delegated Decision (EU) 2024/1441, which establishes a harmonised methodology for their measurement in water intended for human consumption. Furthermore, the recently adopted Urban Waste Water Treatment Directive (EU) 2024/3019 is expected to further strengthen monitoring requirements for wastewater and sludge. In parallel, important standardisation efforts have been developed through ISO 16094-2 for particle-based analysis by micro-FTIR and micro-Raman spectroscopy, and ISO 16094-3 for mass-based thermo-analytical methods such as Py-GC-MS.

This contribution provides an overview of the current regulatory framework, analytical methodologies and international harmonisation activities supporting MPs monitoring in water, including standardisation initiatives, reference materials and inter-laboratory comparison studies. Particular attention will be given to the main methodological challenges that still need to be addressed to ensure reliable, traceable and internationally comparable measurements for future regulatory implementation.

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# TEMPORAL AND SPATIAL TRENDS ASSESSMENT OF PSYCHOACTIVE PHARMACEUTICALS USE IN ITALY

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Wastewater-based epidemiology (WBE) provides an objective and up-to-date approach to assess substance use, such as pharmaceuticals, in a population during specific time periods and across specific geographical areas. The COVID-19 pandemic and associated restrictions had significant short- and long-term effects on mental health, leading the scientific community to investigate their impact on the use of psychoactive pharmaceuticals. This study applied WBE to evaluate the patterns of use of psychoactive pharmaceuticals in Italy during the COVID-19 pandemic and the subsequent period, also performing a two-year study on their spatial distribution in Italy. Biomarkers were selected based on the most used antipsychotics, anti-depressants and anxiolytics in Italy, as reported by the Italian "Agenzia del Farmaco". A specific HPLC-MS/MS method was validated following current directives, ensuring high selectivity and sensitivity for wastewater analysis. Recoveries were higher than 80%, and limits of quantification in wastewater were in the low ng/L range. Mass loads (i.e. concentration multiplied by the daily wastewater flow rate) normalized to population were used as a proxy of use in each community investigated. The use of psychoactive pharmaceuticals was assessed in the city of Milan over the COVID-19 pandemic period (2020-2021) and the subsequent period (2022-2023) and in 20 of the largest Italian cities in January - March 2024 and 2025. 24-h composite raw wastewater samples were collected biweekly in Milan, while the national monitoring campaign was conducted by sampling for seven consecutive days. Results from the four-year monitoring in Milan showed higher loads of almost all investigated pharmaceuticals in 2020-2021, corresponding with the period of tighter restrictions. These trends were followed by a significant decrease of use in the second part of 2021 and in 2022, when restrictions were eased. Results from the first and second national campaign showed different profiles of use, with variations among Italian regions. Results from 2023 (COVID-19 campaign) are currently being analysed, with the aim of assessing whether the decrease in psychoactive pharmaceuticals use observed in 2021 and 2022 was persisting over a longer period post- pandemic. WBE was able to identify specific profiles of use in different communities and highlight temporal trends over specific periods as the COVID-19 pandemic. Results confirm WBE potential as a complementary surveillance tool for assessing human health.

# ADVANCED STRATEGIES FOR CORAL REEF CONSERVATION AND RESTORATION

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Coral reefs are one of the most biodiverse ecosystems in the world and provide support for food, medicine, and tourism to the local population. This fascinating ecosystem is threatened by decline due to climate change and human-related activity. It has been estimated that by the end of 2050, 50% of the reefs will be damaged. Massive events of coral disease outbreaks and coral bleaching are becoming more frequent every year. Researchers are making several efforts in the direction of healing, restoring, and rescuing the reefs, but most of their approaches have been unsuccessful in arresting the progression of this ecological drama. Here, innovative and advanced strategies combining knowledge from materials science, pharmaceutics, marine sciences, and ecology will be presented. The presentation will describe the design, characterization, and application of biomaterials for the underwater delivery of antimicrobial and antioxidant molecules, as well as probiotics, to corals. Conductive and hardening vegetable oil-based pastes for anchoring, transplanting, and restoring corals will also be demonstrated. The biomaterials produced are aligned with the United Nations Sustainable Development Goals and aim to give this millennia-old underwater paradise a chance at survival.

# SYNTHETIC FRAGRANCES IN AQUATIC ECOSYSTEMS: FROM ANALYTICAL CHALLENGES TO ENVIRONMENTAL OCCURRENCE ACROSS MULTIPLE MATRICES

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Synthetic fragrances are widely used in personal care products, detergents, cosmetics, and household formulations, resulting in their continuous release into aquatic environments through wastewater discharges. Despite growing concerns regarding their persistence, bioaccumulation potential, and possible ecotoxicological effects, information on their occurrence and distribution across environmental compartments remains limited. Moreover, the chemical diversity of fragrance compounds and the complexity of environmental matrices pose significant analytical challenges for their reliable determination at trace levels. This study presents the development and application of an analytical workflow for the determination of selected synthetic fragrances in multiple aquatic environmental matrices, including surface water and wastewater, sediments and sludges, and aquatic biota. Sample preparation procedures were optimized according to matrix-specific characteristics, while instrumental analysis was performed using gas chromatography coupled with tandem mass spectrometry (GC-MS/MS), ensuring high sensitivity and selectivity. Methods were applied to samples collected from urban wastewater treatment plants and freshwater ecosystems subjected to different anthropogenic pressures. Results revealed the widespread occurrence of several fragrance compounds, with distinct distribution patterns among matrices, reflecting differences in physicochemical properties and environmental fate. Higher concentrations were generally associated with areas influenced by urban and wastewater inputs, while sediment and biotic samples highlighted the tendency of some compounds to accumulate in environmental compartments other than the water column. These findings contribute to improving current knowledge on the environmental occurrence of synthetic fragrances, highlight key analytical considerations for future environmental monitoring programs and support the inclusion of synthetic fragrances among contaminants of emerging concern requiring further investigation regarding their ecological risks.

# IL CONTROLLO QUALITA' NELLE ACQUE MINERALI: IL RUOLO DEL LABORATORIO UNIVERSITARIO COME PARTNER STRATEGICO. ASPETTI TECNICI E NORMATIVI.

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Il controllo qualità delle acque minerali naturali imbottigliate si configura come un sistema integrato ad elevata complessità, in cui convergono requisiti normativi, metrologici e analitici, finalizzati alla dimostrazione della purezza originaria e della costanza compositiva dell'acqua alla sorgente. Nel contesto regolatorio italiano ed europeo, il quadro definito dal D.Lgs. 176/2011 e dal DM 10 febbraio 2015 stabilisce criteri stringenti per il riconoscimento ministeriale e il mantenimento delle caratteristiche chimico-fisiche e microbiologiche delle acque minerali, introducendo specifici requisiti di prestazione analitica e controlli periodici obbligatori. Il presente contributo analizza il ruolo del laboratorio universitario quale soggetto terzo qualificato nel processo di validazione del dato analitico, con particolare riferimento alla collaborazione tra Acqua Sant'Anna e il Dipartimento di Biotecnologie Molecolari e Scienze per la Salute dell'Università di Torino. Tale sinergia consente di garantire tracciabilità, riproducibilità e valore legale delle determinazioni analitiche, attraverso protocolli ufficiali di campionamento, metodiche ufficiali e verifica metrologica dei risultati. Particolare attenzione è rivolta alle criticità analitiche associate alle acque ultra-leggere, caratterizzate da bassissima conducibilità e concentrazioni di analiti prossime o inferiori ai limiti di rivelabilità. I risultati evidenziano come l'integrazione strutturata tra industria e laboratorio pubblico rappresenti un modello avanzato di assicurazione della qualità, in grado di coniugare rigore scientifico, conformità normativa e tutela del consumatore lungo l'intera filiera, dalla sorgente al prodotto finito.

# BEYOND ROUTINE IDENTIFICATION IN WATER TESTING: HIGH-RESOLUTION ESCHERICHIA COLI SUBTYPING AND PATHOGEN SCREENING USING SHIMADZU MALDI EASYCARE AND MABRITECCENTRAL DB

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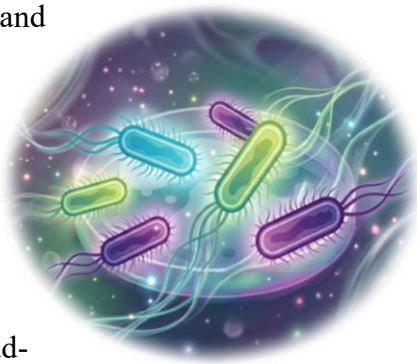
**Abstract**: Microbiological monitoring of water matrices requires rapid, accurate, and sustainable analytical solutions. Traditional MALDI-TOF mass spectrometry exhibits significant limitations when facing critical bacterial complexes; for instance, phylogenetically specular species such as Escherichia coli (the regulatory indicator for fecal contamination) and Shigella (the pathogen responsible for dysentery) often remain indistinguishable using standard databases. To overcome these barriers, this work presents a novel integrated solution arising from the collaboration between Shimadzu and Mabritec. The platform combines the compact hardware of the benchtop Shimadzu MALDI-8020 EasyCare series with the innovative cloud-based Mabriteccentral DB algorithm. Compared to empirical spectra-based databases, this system leverages a genome-guided approach utilizing ribosomal protein markers predicted in silico from over 440,000 complete genomes. Adopting an optimized sample preparation with sinapinic acid matrix and dedicated acquisition methods enables the precise detection of biomarkers in the 4–28 kDa mass range, which are otherwise invisible. Validation data demonstrate the system's effectiveness in addressing water sector challenges: for the Escherichia / Shigella module, the platform clearly discriminates between the two genera, achieving 99.82% specificity and 88.60% sensitivity on Shigella spp. isolates. Concurrently, the system automatically classifies the 7 major phylogenetic lineages of E. coli (A, B1, B2, C, D, E1, E2), showing 94.20% specificity and 82.60% sensitivity across 1,657 strains, thus providing a powerful tool for environmental source tracking.



# AUTOMATION OF THERMO FISHER SCIENTIFIC: FROM SAMPLE PREPARATION TO ADVANCED MASS SPECTROMETRY FOR MORE EFFICIENT WATER ANALYSIS

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Analytical laboratories are increasingly challenged to deliver higher productivity, improved data quality, and faster turnaround times while operating with limited resources and growing sustainability requirements. Automation represents a key strategy to address these demands by reducing manual sample preparation steps, minimizing human error, and allowing skilled personnel to focus on higher-value activities.

This presentation will showcase how Thermo Fisher Scientific automated workflows can support laboratories in optimizing routine and advanced analytical operations, particularly in the field of water analysis. Practical examples will include automated sample preparation solutions for PFAS determination using LC-MS workflows, including automated DLLME (Dispersive Liquid–Liquid MicroExtraction) approaches, as well as Automated SVOC Analysis workflows for GC-MS applications. These solutions can be implemented either as online or offline configurations, providing flexibility according to laboratory requirements and throughput needs.

The presented workflows are designed to seamlessly integrate with Thermo Scientific LC-MS and GC-MS platforms, including both low- and high-resolution mass spectrometer. The combination of intelligent automation, advanced sample preparation, and high-performance mass spectrometry enables laboratories to address increasingly complex analytical challenges while improving robustness, reproducibility, and operational efficiency.

Attendees will discover how adaptable automation platforms can help reduce solvent consumption, support greener laboratory practices, improve analytical throughput, and generate a measurable return on investment. Through real-world examples, this presentation will demonstrate how modern automated workflows can transform laboratory operations, enabling laboratories to achieve more with existing resources while maintaining the highest analytical standards.

# MALDI-TOF MS PROTEOMICS-DRIVEN BIOTYPING OF ARBUSCULAR MYCORRHIZAL FUNGI

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Arbuscular mycorrhizal fungi (AMF) are members of the phylum Glomeromycota [1]. These widespread microorganisms form symbiotic associations with most terrestrial plants. Currently, approximately 355 Glomeromycota species have been documented, with multiple isolates described within individual species. Recent research shows that variability at the isolate level within AM fungal species and genera can significantly enhance plant growth, nutrient uptake, and overall symbiotic efficiency, highlighting the ecological importance of genetic diversity among isolates [2]. Nevertheless, assessing inter-isolate diversity in less-studied lineages such as *Archaeospora* remains challenging due to limited morphological data and insufficient DNA sequence information. One potential tool, Matrix-Assisted Laser Desorption Ionisation Time-of-Flight Mass Spectrometry (MALDI-TOF MS) proteomics-driven biotyping, has been effective in resolving AM fungal taxonomy [3]; however, its application to inter-isolate diversity assessment remains unexplored. To address this gap, in the present study, 15 *Archaeospora* isolates were identified using MALDI-TOF MS, with *Funneliformis mosseae* isolates serving as controls. Proteomics-driven biotyping revealed substantial inter-isolate diversity within *Archaeospora* isolates, as those from geographically close sites exhibited similar proteomic spectra, while isolates from distant locations displayed distinct profiles. In contrast, diversity among *F. mosseae* isolates was comparatively lower. Collectively, these results indicate that MALDI-TOF MS may provide a reliable and rapid alternative to DNA sequencing for isolate-level AMF identification.

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# NEW CHALLENGES FOR WATER QUALITY: EMERGING CONTAMINANTS AND MASS SPECTROMETRY

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The increasing occurrence of emerging contaminants in aquatic environments poses significant challenges for water quality monitoring and public health protection. Among these compounds, per- and polyfluoroalkyl substances (PFAS), including perfluorooctanoic acid (PFOA) and trifluoroacetic acid (TFA), bisphenols, and pharmaceutical residues have attracted growing attention due to their persistence, widespread distribution, and potential adverse effects on ecosystems and human health. Recent advances in analytical chemistry, particularly high-performance liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS), gaschromaotgrafy coupled with mass spectrometry (GC-MS) and high-resolution mass spectrometry (HRMS), have greatly enhanced the capability to detect and quantify these contaminants at trace and ultra-trace levels in complex environmental matrices. These techniques provide the sensitivity, selectivity, and reliability required to support regulatory frameworks and risk assessment strategies. This paper will cover different waters and the regulatory aspects related to the research of emerging contaminants and analyze the mass spectrometry methodologies for the determination of emerging contaminants in waters, highlighting the analytical challenges related to sample preparation and method validation and untargeted screening approaches.

# INTEGRATED BIOLOGICAL – PHOTOCATALYTIC APPROACHES FOR WATER PURIFICATION: PROCESS ORDER, EFFICIENCY, AND TRANSFORMATION PATHWAYS

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Sulfonamide antibiotics such as sulfisoxazole (SSX) are persistent environmental pollutants that are discharged from wastewater treatment plants due to inefficient removal by conventional activated sludge technology and have been detected in various environmental matrices. This study investigated two combined treatment strategies for SSX removal: photocatalysis using TiO2 (PT) as an advanced oxidation process and microalgae-based treatment (AT) as nature-based solution, tested in two sequences: AT/PT and PT/AT. In AT, the concentrations of SSX and its transformation products (TP), as well as microalgal growth parameters (optical density and Chl A), were followed over 8 days under a photoperiod of 16h:8h (light:dark) and constant mixing. In PT, the solutions were treated in a photoreactor under continuous irradiation with UV-A and aeration over 4 hours, during which the concentration of SSX and its TPs were followed. In the AT/PT strategy, the presence of SSX showed no inhibitory effect on the microalgae, with enhanced overall SSX removal in AT/PT compared to PT alone, and three identified TPs. In PT/AT strategy, the photocatalyticaly treated WW alone limited algal growth, likely due to photo-oxidative stress. However, the presence of SSX likely mitigated the stress and enhanced the growth. The most SSX removal occurred during the PT phase, with minimal contribution from subsequent AT. Results indicate overall higher effectiveness of the PT/AT system, with 24% of SSX remaining after treatment, compared to 36% after AT/PT. Algal treatment prior to PT (AT/PT) shows promise for enhancing photocatalytic efficiency and reducing TP accumulation, but offers limited benefits for SSX removal when applied post-PT. Study highlights that sequencing of these technologies matters significantly for treatment effectiveness and byproduct formation.

# POSTER CONTRIBUTIONS

# AUTOMATED HRMS-BASED WORKFLOW FOR SCREENING PHARMACEUTICAL RESIDUES IN SURFACE WATERS

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The widespread occurrence of pharmaceutical pollutants in aquatic ecosystems has become an important environmental concern due to their persistence and ecological effects. Environmental surveillance of pharmaceuticals is essential to evaluate contamination patterns and support water quality monitoring strategies [1].

In this study, an untargeted SPE-HPLC-HRMS was developed for the investigation of pharmaceutical contaminants in surface waters. Sample preparation was carried out using mixed-mode HLB SPE cartridges to improve extraction efficiency. An Orbitrap Fusion Tribrid mass spectrometer operated in both positive and negative ionization modes with full MS and data-dependent MS/MS acquisition was used for analysis. Since the complexity of HRMS datasets, an automated data-processing strategy was implemented. Raw data were pre-processed and molecules were annotated using MZmine (version 3.1.0) [2], while a dedicated Python-based workflow integrated with the DrugBank database (v. 5.1.14) was employed for compound filtering and prioritization.

This pipeline enabled a substantial reduction in manual data handling and improved annotation consistency. The custom workflow was applied to Po River surface water samples collected in Piedmont (Italy) from January to August 2025, leading to the annotation of several pharmaceuticals, including sulfamethoxazole.

Overall, the combination of untargeted approach with automated computational processing provides an effective strategy for environmental surveillance of pharmaceutical pollutants and may support future studies on contamination sources, distribution patterns, and persistence in aquatic systems.

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# MONITORING GLUTATHIONE RELEASE FROM CHITOSAN-BASED NANOPARTICLES USING LC-MS/MS

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Coral reefs, among the most important and biodiverse marine ecosystems worldwide, are currently threatened by a phenomenon known as coral bleaching [1], which represents a stress response to oxidative damage [2]. This stress can be triggered by several environmental factors, including the increase in sea surface temperature. L-Glutathione (GSH), a natural tripeptide with antioxidant properties, exerts a protective effect in cells against oxidative stress [3]. Therefore, its supplementation in corals might represent a promising strategy for mitigating bleaching. On the other hand, GSH's therapeutic potential is limited by its low chemical stability, due to rapid oxidation and dimerization into Glutathione Disulfide (GSSG). Encapsulating GSH into nanoparticles might help control its release in water, thereby slowing its conversion into GSSG. In this context, a chitosan-based delivery system has been developed and evaluated for controlled GSH release.[4]

GSH and GSSG were analyzed using a Waters ACQUITY UPLC-MS/MS system, consisting of a triple quadrupole detector (TQD) mass spectrometer equipped with electrospray ionization (ESI) source and a photodiode array detector (PDA) from Waters Inc. (Milford, MA, USA). The analyses were performed on a ACQUITY UPLC HSS T3 column (100 x 2.1 mm ID, particle size 1.8 μm) using H2O + 0.1% HCOOH (A) and $CH_3CN$ + 0.1% HCOOH (B) as mobile phase. A linear gradient of B in A was applied to separate GSH and GSSG. Both molecules were detected in positive mode, by following their MRM transitions in targeted mode.

We observed a higher incorporation efficiency for GSSG (38.6 ± 3.59 %) compared to GSH (12.3 ± 0.63 %), resulting in a total glutathione encapsulation (as GSH+GSSG) efficiency of 51.2 ± 3.97 % ($n = 4$). Release studies showed a fast release of GSSG in the first 3 hours, with a maximum concentration of 388.2 μM, followed by a slower decay of GSSG concentration in the following hours. In contrast, GSH rapidly decreased to trace levels. These results demonstrate that optimizing encapsulation efficiency within the chitosan system is critical to enhance glutathione stability and to control its release profiles.

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# HPLC-MS MONITORING OF MUCIN-BASED MATERIALS FOR WASTEWATER TREATMENT

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The persistence of pharmaceuticals, pesticides, and other emerging contaminants in wastewater, even at trace levels, represents a major environmental challenge and a significant public health concern. This issue requires the development of innovative and sustainable treatment strategies in order to obtain higher quality water from WWTPs (wastewater treatment plants).[1] In this context, new materials are being developed to improve wastewater purification processes and support circular water reuse, for example in agricultural applications. Among these materials, chitosan beads functionalized with mucin, a highly glycosylated protein naturally present in mucus, have high interest due to their biocompatibility, high surface reactivity, and potential interactions with organic pollutants and microorganisms.[2] In this study, chitosan solutions (3.5% w/w in 0.5 M acetic acid) containing porcine gastric mucin (0.1, 0.2, and 0.5% w/w) were prepared and subsequently converted into hydrogel beads, followed by lyophilization. The molecular characterization of the resulting materials was performed using a bottom-up proteomics workflow. Protein content was initially assessed using a fluorescence-based assay (Qubit fluorometer), while protein extracts were subjected to reduction with dithiotreitol (DTT), alkylation with iodoacetamide (IAA), and tryptic digestion prior to analysis by nano-liquid chromatography coupled to high-resolution mass spectrometry (nanoLC-HRMS) using an Orbitrap Fusion Tribrid mass spectrometer. A purified porcine gastric mucin reference standard was successfully characterized, allowing the identification of MUC5AC and associated mucus proteins. The obtained results demonstrate the potential of high-resolution mass spectrometry for the characterization of mucin-functionalized materials and provide valuable information for the optimization of bio-based adsorbents for advanced wastewater treatment applications, contributing to the development of sustainable technologies for pollutant removal and circular water management.

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# MASS SPECTROMETRY-DRIVEN PLATFORMS FOR TRACKING DRUG AND PROTEIN RELEASE FROM MUCIN NANOPARTICLES

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Mucin nanoparticles represent a versatile, biocompatible delivery system investigated for various biomedical applications, ranging from antimicrobial protection to tissue regeneration. This poster presents different mass spectrometry-driven protocols developed to quantify diverse therapeutic agents, both encapsulated within the nanosystems and during their release profiles. For small molecules, targeted liquid chromatography-mass spectrometry (LC-MS) was employed to quantify specific drugs, including antibacterial agents on functionalized cotton gauzes and the antifungal drug fluconazole within the nanoparticles. Separately, for macromolecular cargo aimed at tissue repair, high-resolution mass spectrometry (HRMS) will be used to monitor the profiles of protein growth factors. In all these different setups, mass spectrometry proves to be the core analytical tool capable of evaluating both encapsulation efficiency and release kinetics for small drugs and large proteins alike. This multi-target analytical framework demonstrates the flexibility of mass spectrometry for the quality control and validation of mucin-based formulations.

# HPLC-HRMS ANALYSIS OF PHARMACEUTICALS WITH ECOTOXIC AND RENAL IMPACT IN PIEDMONT WATER BODIES

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Chronic kidney disease (CKD) is a major public health concern, and environmental factors such as river water contamination may contribute to its onset and progression [1]. Among emerging contaminants (ECs), pharmaceutical compounds are of particular interest because of their persistence in aquatic environments and potential effects on human health [2].

This study aimed to develop a targeted HPLC-HRMS method for the identification of 38 pharmaceutical ECs in surface river waters. The investigated compounds included anticonvulsants (e.g. carbamazepine), antidepressants (e.g. venlafaxine), anti-inflammatory drugs (e.g. prednisolone), diuretics (e.g. indapamide), antibacterials (e.g. ciprofloxacin), and anxiolytics (e.g. diazepam). Special attention was given to prednisolone and indapamide because of their possible role in renal dysfunction and CKD progression.

Surface water samples were collected from the Tanaro, Po, Maira, and Ellero Rivers (Cuneo province, Piedmont, Italy) in February and April 2026, based on epidemiological evidence of CKD incidence in the area. Analytes were extracted by solid phase extraction with HLB cartridges and concentrated 1000-fold. HPLC analyses were performed on an ODS column using water/formic acid (0.1%) and acetonitrile as mobile phases, while HRMS analyses were acquired in both positive and negative ionization modes with dedicated MS/MS experiments.

Several pharmaceuticals, including prednisolone and indapamide, were detected in most samples. Carbamazepine and ketoprofen were identified in Po River samples at concentrations of 3.3 and 2.5 ppt, respectively. Indapamide was quantified at 1.5 and 3.8 ppt in Maira and Po River samples. Prednisolone concentrations remained below the LOQ in all samples except the Tanaro River collected in Carrù (1.6 ppt).

The developed HPLC-HRMS method proved effective for the analysis of pharmaceutical ECs in surface waters. The detection of indapamide and prednisolone supports the need for further monitoring campaigns to confirm these preliminary findings.

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# MASS SPECTROMETRY-BASED PROFILING OF SHORT-CHAIN FATTY ACIDS IN 3D INTESTINAL CULTURE MODELS

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The human gut microbiota is a complex ecosystem whose metabolic outputs play a crucial role in maintaining host homeostasis and systemic health. Among these metabolites, short-chain fatty acids (SCFAs) primarily acetic (AA), propionic (PA), and butyric acid (BA) are crucial biomarkers of microbiota balance, modulating immune responses and energy metabolism. Traditional in vitro culture approaches often fail to replicate the structural and viscoelastic complexity of the intestinal microenvironment. To overcome these limitations, advanced three-dimensional culture platforms, such as the Gut3Gel matrix, have been developed to mimic the in vivo mucus barrier. However, quantifying SCFAs within these highly viscous and complex matrices is analytically challenging due to poor LC-MS ionization efficiency and high GC-MS sample preparation variability. To overcome these limitations, this study aims to develop, optimize, and validate two complementary mass spectrometry (MS) based analytical workflows. We evaluate and compare the performance of two different derivatization strategies N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) for GC-MS and 4-bromo-N-methylbenzylamine (4-BNMA) for LC-MS/MS to ensure robust and sensitive SCFA profiling in both conventional culture media and 3D hydrogel matrices. Fecal samples from healthy donors were incubated; microbial suspensions were inoculated into Brain Heart Infusion (BHI) medium and Gut3Gel matrix under aerobic and anaerobic conditions, with aliquots collected at 0, 24, 48, and 72 hours. For the GC-MS workflow, lyophilized samples were derivatized with BSTFA in dichloromethane at 52 °C for 40 min to obtain volatile trimethylsilyl derivatives. Chromatographic separation and identification were performed using helium as the carrier gas, operating in electron ionization mode (70 eV) with Full Scan for compound identification and extracted ion chromatograms (EICs) for quantification [1]. Alternatively, for the LC-MS/MS workflow, extracted samples were activated via carbodiimide-mediated coupling (EDC) and reacted with 4-BNMA to obtain hydrophobic amide derivatives [2] [3]. This modification enhanced positive electrospray ionization (ESI+) efficiency and introduced a unique $^{79}Br/^{81}Br$ isotopic pattern. Separation was carried out using a C18 column under a water/acetonitrile gradient, with detection performed in Multiple Reaction Monitoring (MRM) mode. Both methods were validated for linearity, precision, LOD/LOQ, recovery, and matrix effects through calibration in matrix, using propionic acid-d5 as the internal

standard. The methodology developed was applied to the analysis of a number of real samples, obtained by culturing bacteria from faecal specimens taken from healthy donors.

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# A 3D BIOINSPIRED MUCUS MODEL TO STUDY GUT-BRAIN MOLECULAR DIALOGUE

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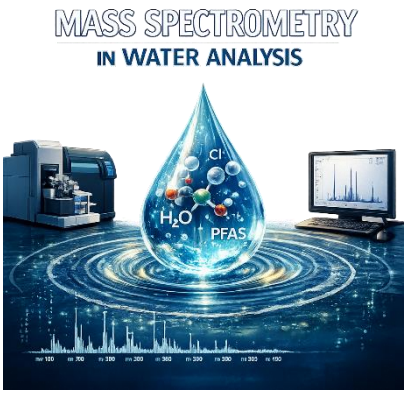
The gut–brain axis relies on a complex molecular dialogue between the gut microbiota and the central nervous system. Although several microbiota-derived metabolites, such as short-chain fatty acids (SCFAs) and indole derivatives, are believed to exert protective effects, their production and transport are often investigated using simplified in vitro models that lack the intestinal mucus layer. In this work, a 3D bioinspired mucus model was developed to better reproduce the physiological intestinal environment and evaluate its impact on microbial metabolite dynamics.




The model was tested using both *Escherichia coli* monocultures and gut microbiota from transgenic Alzheimer's disease mice and non-transgenic controls. Metabolites were quantified by LC-MS in samples collected from the basolateral compartment. Results showed that the presence of the mucus-like 3D structure enhanced the production of beneficial metabolites while simultaneously reducing their diffusion across the barrier compared with conventional planktonic cultures.

These findings demonstrate that mucus plays a crucial role in regulating both microbial metabolism and molecular transport. Therefore, its inclusion is essential for the development of physiologically relevant in vitro models aimed at studying microbiota-derived metabolites and their involvement in gut–brain axis communication.

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# HPLC-MS/MS MONITORING OF TRIFLUOROACETIC ACID (TFA) IN HISTORICAL NEBBIOLO WINE VINTAGES FROM PIEDMONT: SITE-SPECIFIC CONTAMINATION PATTERNS AND TEMPORAL TRENDS

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Trifluoroacetic acid (TFA) represents a significant analytical and environmental concern among emerging contaminants, as it belongs to the class of ultra-short chain perfluoroalkyl substances (USC-PFAS). Its high persistence and strong environmental mobility facilitate its dissemination within agro-food systems and its potential accumulation in plant tissues, with particular relevance for viticultural production chains.

The aim of this study was to apply a validated HPLC-MS/MS method for the quantification of TFA in different vintages of wines produced by two wineries located in the Piedmont region (Italy), specifically in the Gattinara (northeastern Piedmont) area and in the Asti area (southeastern Piedmont). The aims were to characterize the temporal evolution of contamination, to compare concentration levels across vintages and between the two production sites and to search for possible sources of contamination. [1]

TFA was separated and quantified using a high-performance liquid chromatography system coupled to tandem mass spectrometry (HPLC-MS/MS). The chromatographic platform consisted of a Shimadzu Nexera X2 UHPLC system (Shimadzu, Kyoto, Japan), connected to a QTRAP® 5500 triple quadrupole mass spectrometer (Sciex, Darmstadt, Germany). The mass spectrometer was equipped with a Turbo V™ electrospray ionization (ESI) source operating in negative ion mode (ESI-), using nitrogen as nebulizer and desolvation gas, and air as the source of heat. Chromatographic separation was performed using a Luna Polar Pesticides column by Phenomenex (Bologna, Italy) 100 ×2.1 mm, 3 µm particle. A delay column with the same stationary phase (50 ×2.1 mm, 3 µm) was positioned upstream, directly after the HPLC mixer.

The obtained contamination profiles revealed substantial differences in both temporal patterns and quantitative levels of TFA between the two wines analyzed. In Nebbiolo from the Asti area, TFA concentrations exhibited marked interannual variability, characterized by pronounced fluctuations and an overall increasing trend over time. In recent vintages, a sharp increase was observed, reaching a maximum in 2022, when concentrations exceeded 400 µg/L. This pattern suggests an accumulation

process strongly influenced by plant physiological factors and environmental conditions, which may enhance the compound's concentration within grape berries under specific seasonal scenarios.

In contrast, the Gattinara wine, also produced from Nebbiolo grapes, exhibited a distinctly different temporal profile, characterized by a structural discontinuity in the historical series. Prior to 2014, TFA concentrations were close to background levels, with mean values of approximately 14.6 µg/L. From 2014 onwards, a rapid and sustained increase was observed, followed by stabilization at a higher plateau, with a mean concentration of approximately 120.1 µg/L in subsequent vintages. Unlike Nebbiolo from Asti, no marked interannual fluctuations were observed after this shift, suggesting a persistent and structural change in exposure conditions, either within the production chain or the surrounding environmental context. Overall, the results indicate that TFA accumulation in Piedmont wines shows a general increasing trend over time, although with site-specific patterns and markedly different temporal behaviors. Similar increasing trends have also been reported in other European countries, supporting the hypothesis of a broader and progressive increase in environmental TFA contamination.[2] Some analytical evaluation of TFA in soil, water, corks is also presented. This study confirms the suitability of HPLC-MS/MS for the characterization of TFA occurrence patterns in enological matrices, providing valuable insights for environmental monitoring and for the assessment of its distribution in high-quality wine production systems.

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# ONBOARD RESEARCH: HS-SPME-BASED MONITORING OF ORGANIC MICROPOLLUTANTS IN REMOTE MARINE ENVIRONMENTS

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The monitoring of organic pollutants in remote and offshore environments remains a major analytical challenge due to low concentrations and logistical constraints associated with sample collection and preservation [1]. This is particularly relevant since recent global surveys have highlighted a widespread presence of anthropogenic compounds throughout marine dissolved organic matter [2]. In this context, solid-phase microextraction (SPME) represents a promising solvent-free approach that combines simplicity, portability, and high sensitivity [3]. Starting from this basis, we optimized a headspace SPME coupled with triple quadrupole gas chromatography-mass spectrometry (GC-MS/MS) analytical workflow for the monitoring of volatile and semi-volatile organic contaminants in marine waters, with particular focus on polycyclic aromatic hydrocarbons (PAHs), petroleum hydrocarbons, and BTEX. Specifically, we tested three-phase DVB/Carbon-WR/PDMS using a HP-5MS UI (30 m x 0,25 mm I.D. x 0,25 um F.T.) and DB-624 UI (60 m x 0,320 mm I.D. x 1,80 um F.T.). For each target compound we selected two MRM transition for a total of 32 with a cycle time of 400 ms and a cycle per second of 2,5; LOQ calculated from matrix-matched calibration curves ranged from 11.87 pg/mL and 35.98 pg/mL, recoveries (%) from 91,0 to 106,0 and repeatability (RSD) from 6,0 to 8,8. Compound stability test on fiber demonstrated a target analytes loss <5% for 2 weeks storage at 20 °C. We also effectively tested the transport of the SPME fibers between the laboratory and the boat using dedicated storage cases and the application of kinetic calibration [4]. Overall, the developed approach provides a practical framework for oceanographic surveys related to citizen-science initiatives, offering

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opportunities for integration of emerging contaminants measurements in ecosystem health assessment.

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# LC-MS/MS CHARACTERIZATION OF EMERGING CONTAMINANTS IN LIQUID AND SOLID MATRICES OF A CONVENTIONAL WASTEWATER TREATMENT PLANT

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Emerging contaminants (ECs) pose a growing global environmental concern due to their potential adverse effects on biodiversity and human health. Wastewater treatment plants (WWTPs) are the primary receptors for these substances and one of the main pathways into the environment. Therefore, accurately characterizing and quantifying ECs in wastewater is essential to evaluate their distribution and assess whether conventional processes, not originally designed for such micropollutants, can effectively eliminate them to comply with increasingly stringent European Union regulations.

This study analyzed seasonal influent, effluent, and sewage sludge samples from a municipal WWTP in Northern Italy. Target ECs included pharmaceuticals, antimycotics, disinfectants, industrial products, and pesticides. Aqueous samples were filtered and extracted via solid-phase extraction (SPE). Sludge samples were lyophilized, extracted by accelerated solvent extraction (ASE), and purified using SPE. Instrumental analysis was performed via HPLC coupled with a triple quadrupole mass spectrometer. Most ECs were ubiquitous in liquid fractions, with macrolide antibiotics, sartans, anti-inflammatories, diuretics, benzotriazole, imidacloprid, and DEET being the most abundant. Conversely, disinfectants and antimycotics concentrated heavily in sewage sludge. Mass load calculations showed total removal rates mostly (> 80), meeting the EU minimum efficiency standards. However, lower removal rates (< 30) were observed for specific recalcitrant molecules, including certain antibiotics (sulphonamides, erythromycin, clindamycin), other pharmaceuticals (e.g., carbamazepine, venlafaxine, hydrochlorothiazide), and benzotriazole. Disinfectants and antimycotics were removed from the aqueous phase but accumulate in the solid phase of the sludge. Seasonal variability was observed only for pesticides and antibiotics. These findings highlight the need of deeper investigations on a wider perspective, including, in particular, the role of advanced treatment technologies and the effect-based assessment of water/sludge discharge or reuse practice.

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# ASSESSMENT OF ILLICIT DRUG USE AND REMOVAL EFFICIENCY IN ITALIAN WASTEWATER TREATMENT PLANTS THROUGH WASTEWATER-BASED EPIDEMIOLOGY

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Wastewater-Based Epidemiology (WBE) is an analytical-epidemiological approach that relies on the principle that, following consumption, psychoactive substances are excreted partly unchanged and partly as metabolites in urine[1]. These residues enter the sewage system and reach wastewater treatment plants (WWTPs), where samples can be collected and analyzed to quantify drug residues for the estimation of cumulative substance consumption in the population[2].

This study aims to assess the consumption of major drugs of abuse and to evaluate the degradation rates of parent compounds and metabolites in WWTPs.

To estimate drug consumption in 38 Italian cities, 24-hour composite samples were collected at municipal wastewater treatment plants over a week. The study analyzed traditional drugs and new psychoactive substances (NPS), using solid-phase extraction followed by HPLC-MS/MS.

Results showed cannabis as the most used substance, followed by cocaine, heroin, and methamphetamine, while NPS consumption remained low, mainly involving synthetic cathinones and nitrosamines. The analysis of influent and effluent wastewater revealed high removal efficiencies for most of the compounds (86–100%), except for codeine and methamphetamine (73–77%) and methadone (~35%).

Wastewater treatment plants (WWTPs) effectively remove psychoactive substances, though continuous monitoring is crucial due to the continuous use and discharge of these compounds, which can create complex mixtures impacting aquatic life. Moreover, wastewater-based epidemiology (WBE) complements traditional population surveys by providing objective, real-time data and overcoming the limitations of self-reported information.

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