28th BSMS meeting, 21 May 2026 @ VITO (Mol)

PROGRAM

9h00 Coffee & welcome

9h30 Opening

9h40 K01: MS and digitalization in analytical R&D labs: boosting innovation by efficiency and effectiveness enhancement. Ron Peters (Covestro)

10h20 L01: Mass spectrometry, a powerful analytical tool for textile characterization, enabling the investigation of both released polymers and coating materials from textiles. Xiaoyu Zhang (VITO)

10h40 L02: Limitations of TIMS TOF instruments and trade-off solutions to develop methods for analytical and physical chemistry using electrospray, atmospheric pressure chemical ionization, and MALDI ion sources for a low to medium mass range. Johann Far (ULiège)

11h00 Coffee & networking

11h30 Flash presentations (2 min/speaker – best poster award candidates)

11h50 Sponsor presentations (5 min/speaker)
Agilent / Bruker / IonBench / msVision / NaturaTech / Sciex / Thermo Fisher / Waters

12h35 Lunch, networking & posters

13h50 K02: What's in the water? Challenges and opportunities of water quality monitoring with non-target screening. Frederic Béen (KWR)

14h30 L03: From 4-MMC to 2-MMC: wastewater-based evidence of an isomeric shift in methylmethcathinone use in Belgium. Natan Van Wichelen (UAntwerpen)

14h50 Coffee & networking

15h25 L04: Analysing biomolecules with combined flow-induced dispersion and native mass spectrometry. Frederik Lermyte (UAntwerpen)

15h45 L05: Probing photoswitchable azobenzene-functionalized macrocyclic peptoids by mass spectrometry. Ruth Stella Kamguem Kamga (UMons)

16h05 Poster prize + closing

16h15 Guided tour at the VITO laboratories

17h00 Farewell

MS and digitalization in analytical R&D labs: boosting innovation by efficiency and effectiveness enhancement

K01

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Covestro is a world-leading manufacturer of high-quality polymer materials, driving the vision to become fully circular. In this transition, analytical R&D labs must evolve to increase both efficiency and effectiveness. This lecture discusses the modular building blocks we are developing to integrate digitalization and mass spectrometry (MS) into a future automated ecosystem. I will shortly introduce some individual key components of our digital architecture designed to bridge the gap between chemical synthesis and structural characterization. This includes the RoboChem platform for automated synthesis optimization based on Bayesian optimisation and the ASSIST robotics platform for automated sample preparation. While these currently applied as powerful standalone pillars of automation, we working to connect these units through an AMR to have a fully automated R&D workflow. To extract maximum information from these emerging workflows, I will show some advanced MS strategies that expand the analytical window for polymer analysis. We demonstrate how to significantly extend the detectable molecular-weight range and structural resolution for complex polymers using techniques such as supercharging MS, 2DLC-MS, and SEC-PyrMS. Finally, we show how these MS based set-ups combined with advanced data analysis (based on machine learning concepts) is being applied to obtain new insights, such as polymer branching and polymer sequence information.

What's in the water?
Challenges and opportunities of water quality monitoring with non-target screening

K02

Frederic Béen

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Water resources are increasingly under pressure due to climate change and human activities. The emission of a broad, often unknown range of chemicals presents significant challenges for drinking- and wastewater utilities, environmental laboratories, and regulators. These chemicals include pharmaceuticals, pesticides, biocides, industrial compounds, and personal care products. Adding to the complexity, many of these chemicals undergo transformations in the environment or during treatment, further expanding the chemical space that needs to be monitored. Conventional targeted analytical techniques are insufficient to track the vast array of potential chemicals. High-resolution mass spectrometry (HRMS) has become the method of choice as it allows for the detection of a broader range of chemicals in a single analysis. However, HRMS alone is not enough. Environmental analytical chemists are increasingly combining HRMS with complementary techniques such as orthogonal chromatography, effect-based methods, and advanced data science applications. Notably, the introduction of data visualization and machine learning-based prioritization strategies, which aim to reduce the volume of chemical information and focus on relevant contaminants, is revolutionizing water quality monitoring. The goal of this presentation is to provide an overview of the latest advances in HRMS-based water monitoring strategies, including the integration of complementary techniques and data-driven approaches.

Mass spectrometry, a powerful analytical tool for textile characterization, enabling the investigation of both released polymers and coating materials from textiles

L01

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Textile materials present analytical challenges that span multiple chemical and structural levels, requiring versatile and complementary analytical techniques for comprehensive characterization. In this work, mass spectrometry is applied as a powerful tool to investigate textiles from two distinct yet related perspectives: polymer release during washing and chemical characterization of coating materials.

In the first approach, pyrolysis–gas chromatography–mass spectrometry (Py-GC-MS) was employed for the identification and quantification of polymer particles released from pristine textile materials during washing experiments. This technique enables analysis of polymer composition through characteristic pyrolysis products, allowing differentiation between textile types such as polyethylene terephthalate (PET) and polylactic acid (PLA). The results provide insights into the extent of polymer release and highlight differences in material stability under washing conditions.

In the second approach, liquid chromatography–mass spectrometry (LC-MS) was used to characterize coating materials applied to textiles, with particular focus on soybean oil- and acrylic acid-based coatings (PFAS-free). Both electrospray ionization (ESI) and atmospheric pressure photoionization (APPI) were utilized to broaden the detectable chemical space, targeting different coating materials, respectively. This complementary ionization strategy enabled detailed investigation of the chemical composition of coatings and the evaluation of structural changes induced by plasma treatment. Variations in molecular profiles indicate potential oxidation, fragmentation, and formation of new chemical species following plasma exposure, and with this information, risk assessment of coated textiles can be reached.

Although these two studies address different aspects of textile analysis, together they demonstrate the versatility of mass spectrometry in tackling complex material systems. Py-GC-MS provides robust information on polymer identity and release behavior, while LC-MS offers molecular-level insights into surface chemistry and coating transformations. The combined use of different MS techniques and ionization methods highlights the importance of selecting appropriate analytical strategies depending on the specific research question.

Overall, this work underscores the value of mass spectrometry as a comprehensive analytical toolbox for textile research, enabling improved understanding of both material performance and chemical composition. Such approaches are essential for advancing the assessment of textile durability, functionality, and environmental impact.

Limitations of TIMS TOF instruments and trade-off solutions to develop methods for analytical and physical chemistry using electrospray, atmospheric pressure chemical ionization, and MALDI ion sources for a low to medium mass range

L02

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Electrospray fitted to trapped ion mobility mass spectrometry coupled with time of flight mass analyzer (TIMS TOF) instruments is mainly used for analytical purposes of peptides (bottom up proteomics), lipids (lipidomics), and metabolites (metabolomics).

Compared to ion mobility instruments commercially available, especially the traveling waves ion mobility mass spectrometers from the Waters Synapt G2 series, the Bruker TIMS TOF encounters some instrumental limitations affecting the design experiments, including experimental physical chemistry and analytical applications. The users face the poor efficiency of low mass ion transmission when dealing with relatively large ranges of m/z and collision cross section (CCS). In addition, the range of ion mobility coefficient K (1/K 0 for the TIMS) barely fits with the needs for CCS of single charge state of small or larger ions produced by matrix assisted LASER desorption ionization (MALDI) or LDI. Nevertheless, it is possible to overcome much of these limitations by modifying parameters accessible to the standard users while accepting some trade-offs. One of them is the adjustment of the gas pressure in the TIMS-In region of the instrument, which has a direct impact on ion transmission of low to high mass. This is also mandatory to extend the 1/K 0 range to be compatible with the CCS of singly charged at low or middle/high mass range. The use of stepped voltage for the TIMS and the collision cells also help to improve the ion transmission.

Finally, the latest hardware improvements of the TIMS TOF instrument, such as Ion Charge Control (ICC), also Mobility Range Enhanced (MoRE) and Athena Ion Processor (AIP) can positively impact the general performance of the instruments, especially when the detection of ions from low to middle mass range at once is required. Some practical examples from our group and the literature about the parameters to be changed to overcome some of these limitations will be provided. These include the use of gas chromatography coupled with TIMS TOF for dioxines and polychlorinated biphenyl (PCBs), the use of the Smartbeam 3D from the dual combi-source ESI/MALDI of the timsTOF fleX for MALDI MS experiments applied to oligosaccharides ($1 \leq n \leq 6$), N-glycans, peptides, intact oligonucleotides ($8 \leq mers \leq 30$), and proteins (≤20KDa). The issue for synchronizing the pulsed ion source with the TIMS accumulation and elutions during MALDI, MALDI In-Source Decay (MALDI-ISD), and MALDI Mass Spectrometry Imaging (MALDI MSI) experiments concomitantly with ion mobility separation will be covered as this strongly restricts the experimental design.

From 4-MMC to 2-MMC: wastewater-based evidence of an isomeric shift in methylmethcathinone use in Belgium

L03

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Introduction: Synthetic cathinones are the second largest group of new psychoactive substances (NPS) monitored by the European Union Drugs Agency, with increasing methylmethcathinone (MMC) seizures and self-reported consumption in surveys. However, assessing community-level consumption remains challenging due to the rapidly evolving NPS market, varying legislation, and frequent unintentional use of mislabelled substances. In addition, chromatographic separation of the three MMC isomers (i.e., 2-, 3-, and 4-MMC) remains analytically challenging, yet is essential given their distinct pharmacological profiles and regulatory statuses. This study applied wastewater-based epidemiology (WBE) in Belgium to investigate spatio-temporal trends in MMC consumption.

Materials and methods: An analytical workflow based on solid-phase extraction and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) was developed and validated for the separation and measurement of 2-, 3- and 4-MMC in influent wastewater (IWW). Daily 24-h composite IWW samples were collected from ten locations across northern Belgium (Flanders and the Brussels region) in 2023 and 2025 (n = 311). Additional samples collected between 2019–2022 and in 2024 were analysed for Brussels and Antwerp. Measured concentrations in IWW were transformed to population-normalised mass loads (PNML, expressed in mg/day/1000 inhabitants), accounting for wastewater flow and the population served by the wastewater treatment plant catchment area. Therefore, these PNMLs serve as a proxy for MMC consumption.

Results: MMC use is widespread across Flanders, occurring in both major urban centres and smaller municipalities. Increasing consumption trends were observed over the study period. Furthermore, an isomeric shift in MMC consumption was observed over time. Although 4-MMC was historically the predominant MMC isomer following its emergence around 2010, only minor levels were detected in this study. In contrast, 3-MMC was the dominant isomer in 2023, whereas by 2025 its consumption had declined substantially alongside a marked increase in 2-MMC in all monitored locations. This could be linked to legislative changes in Belgium and the Netherlands.

Conclusion: This study illustrates the strengths of wastewater-based epidemiology to provide near real-time, objective measurements of illicit drug consumption. A rapidly evolving MMC market in Belgium is revealed, with increasing consumption and a clear isomeric shift from 3-MMC to 2-MMC, potentially linked to legislative changes. These findings highlight the importance of monitoring all MMC isomers to draw correct conclusions on MMC consumption patterns, which can be used to support evidence-based drug policy (re-)evaluation.

Analysing biomolecules with combined flow-induced dispersion and native mass spectrometry

L04

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Tremendous progress has been made in biological science as a result of protein mass spectrometry; however, there is still a pressing need for further methodological improvements. Particularly exciting developments have been made in recent years in the field of native mass spectrometry, in which noncovalent protein complexes remain bound during ionisation and transfer into the gas phase. Through measurement of the mass of the intact complex(es), possibly augmented with gas-phase fragmentation and mass measurement of the resulting products, important insights have been obtained in the stoichiometry and stability of protein-protein complexes, as well as (for example) binding of natural or artificial ligands, and the action of novel drug moieties such as molecular glues, which selectively modulate protein-protein interactions. Despite this tremendous potential, widespread adoption of native MS is hindered by its incompatibility with non-volatile salts and traditional biological buffers, as these compounds lead to loss of sensitivity and extensive adduct formation, with peak broadening and low signal-to-noise ratios as a result. This fact typically necessitates a separate sample preparation workflow to other analysis methods; furthermore, the use of non-traditional buffers raises questions about how biologically relevant results are, especially if proteins spend a long time in such buffers. Here, we present a versatile approach that exploits dispersion physics within a laminar flow through a capillary to separate species based on their hydrodynamic radii. In one implementation, we achieved ultrafast buffer exchange of proteins, protein-protein and protein-ligand complexes, and double-stranded DNA from traditional, high-salt buffers. Signals were detected in just 30 seconds and each experiment took approximately 2 minutes in total. In a different variant, exploiting Taylor dispersion, experiments typically still took less than 10 minutes, but allowed for simultaneous buffer exchange and hydrodynamic radius measurement of analytes. These methods use only aqueous solutions, are flexible and easily adopted, and can be tuned to the desired information and available equipment.

Probing photoswitchable azobenzene-functionalized macrocyclic peptoids by mass spectrometry

L05

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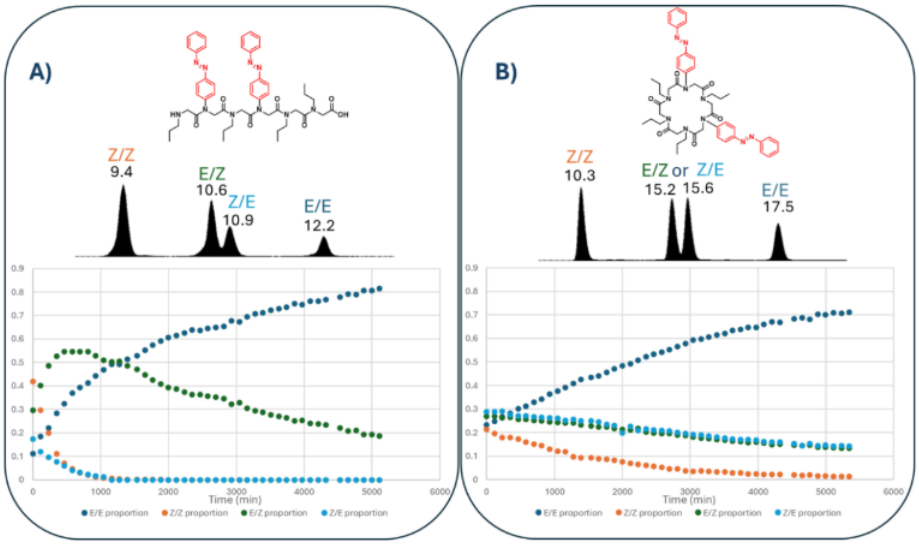
Azobenzenes (**AB**) constitute a particularly interesting class of molecular photoswitches due to their ability to switch reversibly between their trans (E) and cis (Z) configurations under the influence of light. This property is exploited in many fields, including solar energy storage, smart materials and pharmacology. Indeed, due to the reversible photoisomerization and back isomerization processes, the physico-chemical properties of azobenzene congeners may be dynamically modified on the way to innovative applications such as light-activable sensors, organic transistors and controlled drug release [1], [2], [3]. However, pristine **AB** absorbs in the UV range, limiting e.g. biomedical applications due to low tissue penetration and phototoxicity of UV light. Additionally, the spectral overlap between the E and Z isomers limits the E-to-Z conversion. The thermal half-life ($t_{1/2}$) of the Z-isomer is another tuneable aspect that must be optimized, upon molecular engineering, for targeted applications.

Strategies for modulating azobenzenes properties include incorporating them into linear macromolecules, such as peptoids. Our group recently demonstrated that the half-life (t1/2) of the Z-isomer is significantly affected by the anchoring site [4], and we here hypothesized that cyclization would also affect these properties due to the constraints imposed by the ring structure. The enhanced stability and structural modularity of cyclic peptoids would provide an ideal platform for harnessing azobenzene properties while introducing functional diversity.

This work aims to elucidate how the cyclization of peptoids bearing 2 azobenzene units affects their properties, and how their relative positioning within the macrocycle influences these properties, compared to a peptoid bearing a single azobenzene unit. The synthesized molecules were characterized by MS, MS/MS, LC–MS, and cyclic IMS–MS. We present LC– MS as the primary tool for studying the isomers of these peptoids and for determining the half-lives. The chromatogram of a peptoid functionalized with two azobenzene units displays four peaks assigned to the Z/Z, Z/E, E/Z, and E/E isomers. LC-MS results highlight distinct behaviours of the two azobenzene units in the linear peptoid, in contrast to the cyclic analogue.



LC–MS chromatograms and evolution of the relative proportions of Z/Z, E/Z, Z/E, and E/E isomers for the peptoid sequence NpropNazoNpropNazoNpropNpropOH: (A) linear form, (B) cyclic form. Analysis performed by HPLC-MS (C18 Agilent Eclipse plus column, gradient H2O (0.01% HCOOH)/ACN, 90/10 to 0/100).

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Self-assembled monolayers of azobenzene derivatives: on the use of mass spectrometry to monitor photo and back isomerization reactions

P01

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Photoswitches constitute one of the most ubiquitous and versatile classes of stimuli- responsive molecular systems. A fundamental prerequisite for integrating photoswitchable moieties into adaptive materials is a detailed understanding of their switching behavior across different environments. Among these, azobenzenes (AZO) undergo reversible photoisomerization between the ground-state trans (E) configuration and the metastable cis (Z) configuration. Pristine AZO and many of its derivatives typically absorb in the UV region to induce E-to-Z isomerization, whereas the reverse Z-to-E process is generally triggered by visible light. Despite being among the most extensively studied photoswitches, azobenzenes still suffer from limitations related to incomplete photoisomerization yields and weak thermal half-life of the Z-isomers upon red-shifting the absorbance to match solar irradiation or for biomedical applications.

Two strategies are employed in my project to improve properties of azobenzene for specific applications, with special attention paid at red-shifting the absorbance while conserving a useful half-life time for the Z-isomers. Our workflow relies on (i) synthesizing azobenzenes substituted with donor/acceptor groups and/or replaced by heterocycles for red shifting the absorbance, and (ii) anchoring these molecules on surfaces as self-assembled monolayers (SAMs). Indeed, densely packed SAMs of azobenzene derivatives on rigid gold substrates exhibit a high yield of photoisomerization (>96%) due to cooperative switching effects. In the present research, we intend to graft azobenzene derivatives onto glass surfaces (cheap and transparent) using a click reaction between azobenzene bearing an alkyne group and grafted silane, end-substituted by an azide group.

The determination of the properties of the isolated chromophores is initially performed, in solution to determine the chromophore photoswitching properties as reference data, before evaluating the influence of the intermolecular interactions within the close-packed assemblies, on the substrate. To separate and quantify stereoisomers, LC–MS analyses are used to determine the so-called photostationary state (PSS) in a given solvent, that is the maximum Z-to-E ratio under irradiation. Further, Z-to-E thermal relaxation is monitored via repeated LC–MS analyses at controlled temperature. Extracted Ion Current (EIC) chromatograms are used to monitor stable and metastable isomeric ions and to evaluate the time-dependent evolution of their relative abundances. E and Z isomers are mainly observed as protonated species ([M+H]+) with different ionization yields, as determined by comparison with NMR data at the PSS. A correcting factor was determined and applied in the LC-MS analysis.

Untargeted brain metabolomic profiling of plasma samples using supercritical fluid chromatography–mass spectrometry

P02

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Untargeted metabolomics measures small molecules (<1500 Da) in complex biological matrices, enabling metabolic profiling without prior hypotheses. This approach facilitates the comparison between healthy and diseased states, providing insight into underlying biological processes and supporting the identification of potential biomarkers. Liquid chromatography coupled to mass spectrometry (LC-MS) is widely used to generate metabolic profiles. An alternative to LC is supercritical fluid chromatography (SFC), an efficient separation technique that uses supercritical fluids with low viscosity and high diffusivity as mobile phases, allowing rapid and high-resolution separations. To extend the range of analysable compounds, a unified chromatography (UC)[1] approach, where a gradual transition from a supercritical to liquid mobile phase is made during the analysis, is sometimes applied. Hyphenating SFC with MS enhances detection capabilities and provides better sensitivity and selectivity [2].

This work aims to establish a robust, untargeted SFC-MS method for the metabolic profiling of plasma samples to identify alterations in metabolites associated with neurological disorders.

For method development, a mixture of 52 neurologically relevant metabolites, covering a wide log P range from −5 to 10, was prepared. Seven dissimilar stationary phases[3] were systematically screened using a 65 min linear gradient ranging from 2% till 100% co-solvent. The co-solvent consisted of methanol with 3% water and either ammonium formate, ammonium acetate, ammonium hydroxide, or a combination of ammonium fluoride/ammonium formate as additives. Next, the mobile phase composition was further optimized using a face-centred central composite design with three factors: 1) the ammonium formate concentration, 2) the percentage water content, and 3) the concentration of ammonium fluoride in the co-solvent. Optimal conditions were selected primarily based on the number of separated peaks, but also peak shape, retention behaviour, repeatability and MS response were considered.

The best metabolite separation was obtained on a diol stationary phase using methanol as co-solvent, containing 20 mM ammonium formate, 0.5 mM ammonium fluoride, and 3% water. Using these conditions, 45 compounds were fully or partially separated within 35 minutes (60% co-solvent). Current work focuses on evaluating the necessity of using UC conditions. Avoiding UC conditions could reduce analysis time and lowers the maximum pressure, allowing the use of higher flow rates. Afterwards, the optimal pretreatment step for plasma samples will be selected. Finally, the developed method will be applied to compare plasma samples from neurologically diseased and healthy individuals.

References

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Structural analysis of self-assembled cages by mass specrometry

P03

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In nature, many chemical reactions are catalysed by enzymes through highly specific interactions within a catalytic pocket, which isolates the reactants from their environment and provides a confined reaction space. This remarkable property has stimulated significant interest in the development of 'molecular containers', where the objective is to reproduce such confinement effects in synthetic systems and apply them to molecular encapsulation and reaction catalysis.[1]

Supramolecular cages have attracted particular attention due to their ability to encapsulate different guests such as photoswitchable molecules, thereby impacting the isomerisation kinetics.[2] Typical examples are self-assembled Pd6L4 and Zn4L4 structures, i.e. coordination hosts with specific architectures designed around metal nodes (Pd^{2+} or Zn^{2+}) and organic panels (L), presenting cage structures that may efficiently protect their guests from the environmement.[3,4] The success of the cage self-assembly and of the guest encapsulation are most of the time evaluated by NMR spectroscopy.

We here use ion mobility spectrometry and collision-induced dissociation experiments to analyse in details such supramolecular systems. By measuring collision cross sections, IMS affords invaluable data on the cage 3D structures and on the impact of guest encapsulation on their structures. In particular, high resolution cyclic
ion mobility experiments were used to separate different isomeric forms for specific macro- ions, suggesting the formation of coordination cages with different ligand configurations. In this context, we investigated the self-assembly and self-sorting of Pd(II) coordination cages differing by ligand geometries. Our results provide an alternative to traditional characterization techniques, such as NMR spectroscopy.

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Mitigating aspecific adsorption for sensitive LC-MS/MS analysis of neuromedin U

P04

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Neuromedin U (NmU) is a 2.7 kDa neuropeptide of the neuromedin family that plays a role in the stress response, feeding behavior and smooth muscle contraction. To further explore the involvement of NmU in pathophysiological processes, accurate monitoring of its concentration is essential. To achieve this, a miniaturized UHPLC M-Class system fitted with a Symmetry® C18 trap column and a CSH C18 iKey separation device is used, coupled to a Xevo TQ-XS triple quadrupole mass spectrometer (all from Waters). The system operates in a trap-and-back-flush elution configuration.

Aspecific adsorption represents a major limitation for the sensitive analysis of peptides by liquid chromatography tandem mass spectrometry (LC-MS/MS). Peptides readily bind non-specifically to surfaces, such as Eppendorf tubes, vials, pipette tips and the tubing of the LC system, thereby compromising accurate quantification. In this study, strategies to reduce aspecific adsorption of NmU were evaluated at both the sample preparation and LC level.

To minimize aspecific adsorption, solvents with varying ratios of water and organic modifier were assessed for dissolution of the lyophilized NmU standard and for subsequent dilution steps. The highest AUCs were obtained when NmU was dissolved in water/acetonitrile/formic acid (70:30:0.1%, v:v:v), with all further dilutions prepared with the same solvent. The signal was further enhanced by adding 10% (v:v) acetonitrile and 10% (v:v) formic acid to the sample in Quanrecovery UHPLC vials prior to injection. In addition, passivation of the LC system proved essential and was achieved by repeated injection of 40 µg/mL bovine serum albumin solution, which acts as an adsorption competitor to NmU and saturates non-specific binding sites within the system.

After optimisation of the sample preparation, the UHPLC conditions (mobile phase gradient, column temperature and trapping flow rate, and duration) were investigated to further improve sensitivity and reduce carryover. Optimal performance was achieved using a gradient starting at 5% organic modifier and a column temperature of 45°C. For peptide trapping and subsequent back-flush elution on the trapping column, a flow rate of 10 µL/min and a trapping volume of 30 µL were selected. Using the optimized method, the limit of detection in aqueous standard solutions was in the 100 pM range.

Finally, microdialysis samples were spiked with NmU at different concentrations to compare the sensitivity of the analytical method in water and in microdialysate. Consistently lower peak areas were observed in microdialysis samples, indicating that further methodological improvements are required for reliable

Studying the photoswitching behaviour of NBD to QC by UPLC and cyclic ion mobility mass spectrometry

P05

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In the shift towards an increasing dependence on renewable energy sources, solar energy and its storage play a crucial role in shaping the future of energy management. Molecular solar thermal systems (MOST) are photoswitches capable of undergoing photoisomerization to a higher energy state in which solar energy can be stored. The research presented here focuses on a particular type of photoswitch called nobornadiene (NBD) which can isomerize into quadricyclane (QC) upon irradiation. NBD is quite appealing in terms of photostability and QC is characterised by a high energy density making this isomer couple stand out in the MOST field.

Despite the possibility to assess the properties of photoswitches using mass spectrometry (MS) coupled with different separation techniques such as liquid chromatography or ion mobility, the number of studies about the NBD/QC system in MS remain scarce.

The study reveals that the separation of new NBDs and their respective QCs is efficiently performed using both UPLC and IM coupled with MS. In particular, whereas the LC separation is based on the difference of polarities between NBD/QC, IMS is here challenged for the separation between NBD and QC due to weak internal modifications in geometry between both isomers. In that regard, linear as well as cyclic ion mobility are used and compared for the study of photoswitching between NBD and QC.

Comparison of two chiral derivatization reagents for the enantioselective analysis of amino acids through UHPLC-MS/MS

P06

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The quantification of metabolites, with roles as neurotransmitters, gliotransmitters, or neuromodulators, in the healthy and diseased brain is essential for elucidating the underlying mechanisms of neurological disorders. Among these metabolites, the D-enantiomers of amino acids have gained interest as potential biomarkers in cognitive and neurodegenerative disorders. However, their quantification in mammalian tissues and fluids remains challenging, because of their trace concentrations and the need for robust enantioselective discrimination for each enantiomeric pair.

In this study, two chiral reagents were evaluated, i.e. diacetyl-L-tartaric anhydride (DATAN) and its dibenzoyl derivative (DBTAN), exploiting the ability to separate the derivatized D/L amino acid pairs on a C18 stationary phase, using ultra-high-performance liquid chromatography - electrospray ionization - tandem mass spectrometry (UHPLC-ESI- MS/MS). The pre-column derivatization step with an enantiopure chiral reagent enables the conversion of enantiomers into diastereomers, while enhancing both sensitivity and metabolite stability. Moreover, the influence of the mobile phase composition on the chiral separations was investigated.

Derivatization was carried out using (+)-DATAN or (+)-DBTAN on dried standard solutions of 20 chiral proteinogenic amino acids, incubated at 75°C for two hours. Analysis was performed in positive ionization mode using a UHPLC system coupled to a Xevo TQ-MS (Waters) and equipped with an Acquity BEH C18 column (2.1 mm × 100 mm, 1.7 µm, 130 Å) at a flow rate of 0.3 mL/min and a column temperature of 60°C. Cone and capillary voltages and collision energy were set at 20V, 3kV, and 20eV, respectively, while MRM transitions were optimized for each analyte. The effect of the choice of chiral reagent and mobile phase composition on the resolution of the derivatized D/L amino acids was studied. The aqueous phase was composed of either 0.1% formic acid (A1) or 10 mM ammonium formate with 0.06% formic acid (A2); the organic phase of acetonitrile (B1) or methanol (B2), both with 0.1% formic acid.

The results show that the A2B1 mobile-phase combination yielded the best performance in terms of chiral resolution. Comparing the two reagents, DBTAN provided better chromatographic retention, enabling the use of a higher organic content for the elution, which led to an improvement in the droplet desolvation and ionization efficiency in the ESI source. Among the tested amino acids, ten showed successful baseline separation, seven were partially resolved, whereas three exhibited co-elution of the diastereomers.

To further improve the separation of the amino acids, alternative stationary phase chemistries are currently under investigation.

Cyclic ion mobility spectrometry as an efficient tool for thigh-throughput characterization of combinatorially synthesized peptoids

P07

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Natural metalloenzymes are known to be among the most efficient catalytic systems, enabling complex chemical transformations under mild conditions with remarkable selectivity. Their performance arises from a highly organized three-dimensional structure in which a catalytic metal center is embedded within a precisely defined molecular environment. However, natural enzymes are often working under soft conditions. To overcome this limitation in application range, a minimalistic strategy focusing on the first coordination sphere is adopted, in which artificial catalysts are designed using sequence-defined peptoids that reproduce key features of enzyme active sites. Within these molecules, monomers are incorporated to mimic amino acids involved in metal coordination, with the goal of achieving stronger and more well-defined metal coordination to enhance catalytic activity.

In order to identify the optimal sequence, solid-phase combinatorial chemistry, and more specifically the split-and-mix method, is employed to generate libraries containing all the possible sequences. As a first approach, two different monomer units, i.e. histamine and propylamine, as respectively metal ion binding and non-bulky side chains, are considered. When targeting 4-unit peptoids, 16 different sequences are theoretically present, requiring a high throughput and efficient analytical method to discriminate and identify them. We here develop LC-MS, LC-MSMS and LC-IMS as a powerful combination to solve the structural complexity inherent to combinatorial synthesis. Due to the broad polarity window cover by the peptoid congeners, from full histamine to full propylamine, different HPLC columns are tested, with to date limited success. On the other hand, implementing high resolution cyclic ion mobility spectrometry in the analytical pipeline is demonstrated successful in separating peptoid ions characterized by different sequences.

Step-by-step method optimization with standard proteins for structural characterization of therapeutic megadalton virus-like particles

P08

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Introduction: ADDomers are self-assembling dodecahedral virus-like articles (VLPs) derived from adenovirus, that represent a promising new synthetic antivenom platform to solve issues of snakebite envenomation (a neglected tropical disease[1]) due to their high stability, multivalency, and cost-efficiency . Their structural characterization remains challenging. This work introduces a native Mass Spectrometry (MS) and Ion Mobility (IM-MS) workflow to monitor the assembly kinetics and conformational stability of such 3.5 MDa complexes.

Methods: A modified Synapt G2-S (Waters) for high mass capabilities and native MS and Collision-Induced Unfolding (CIU) experiments was used on model proteins (Myoglobin, Insulin, and bovine serum albumin as BSA) in non-denaturing and denaturing conditions. Energy-resolved collision induced dissociation (or breakdown curves survival yield (SY) experiments) were acquired to determine the relative stability of the molecular assemblies (V50 values as voltage where 50% of SY of the assemblies).

Main results: Training and validation using Holo-Myoglobin $[M+9H^+]^{9+}$ demonstrated stable ion beam in non-denaturing MS conditions. CIU heatmaps showed three specific gas-phase population of conformation. Breakdown curves experiments and survival yield of insulin were successfully determined. Additionally, BSA native mass spectra in reduced and Oxidized forms identified overalkylation because of a carbamidomethylation patterns of +2 kDa (Corresponding to the 35 residues submitted to alkylation for 35 cysteines in BSA sequence). IM-MS and CIU will be applied to protein models of increasing mass before working with ADDomers for mapping their supramolecular assembly and avidity for toxins.

(1) Neglected tropical diseases -- GLOBAL. https://www.who.int/health-topics/neglected-tropical-diseases (accessed 2026-02-14).

Rapid screening of plastic additives in wastewater using DART-MS/MS

P09

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Plastics contain not only polymer materials but also a wide range of additives, designed to improve material characteristics such as flexibility, heat resistance and durability [1] . Common examples include plasticisers, flame retardants, or antioxidants. Such additives are often not chemically bonded to the material, which facilitates their release into the environment. Wastewater treatment plants (WWTPs) play a key role in contaminant removal, yet numerous chemicals persist through treatment processes and may accumulate in terrestrial and aquatic ecosystems.

Direct analysis in real time (DART) is an ambient plasma ionization method that allows for a rapid, direct measurement of diverse chemicals in complex matrices [2] . In this study, DART coupled to high-resolution mass spectrometry was applied to identify plastic-related leachates in wastewater (WW) effluent samples (n=4) from a municipal WWTP. Samples were preconcentrated using stir bar sorptive extraction (Gerstel Twister). A procedural blank (Mili-Q) was included in the analysis to monitor background contamination. A JumpShot DART was coupled with a TimsTOF Pro 2 operated in Auto MS/MS mode, which included a targeted mass list of approximately 200 compounds from an in-house library. Analyses were performed in both positive and negative ionisation mode.

Across all samples, 15 plasticassociated chemicals were detected, including flame retardants, plasticisers, antioxidants, pesticides, and industrial chemicals. Four of those could be further confirmed by MS2 spectra, and two – acetyl tributyl citrate and tributyl phosphate – using reference standards.

Although preliminary, these results demonstrate that DARTMS/MS offers a fast, efficient, and innovative approach for the screening of plastic leachates in WW. The technique allows for rapid screening and subsequent prioritisation of contaminants of interest, showing strong potential for future monitoring applications following further optimisation and validation.

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Acknowledgements:

Financial support was obtained from the European Union's HORIZON EUROPE innovation program, Upstream, under Grant Agreement No. 101112877.

Multi-dimensional analytical approach for the identification of bio-active peptides

P10

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In recent years, food-derived protein hydrolysates have attracted great interest. This is caused by some peptides released from unspecific enzymatic digestion having been found to exhibit biological activities such as antioxidative, anti-inflammatory, and antihypertensive.

Non-specific digestion, notably when non using any intrants such as reduction and alkylating reagents, substantially increases the variety of peptide sequences that can be produced from a protein source. The complexity of the hydrolysate also increases the difficulty of characterizing the sequences of these peptides, especially for those displaying bioactivity due to the lack of standardized methods and bioinformatic workflow.

To alleviate this drawback, we propose to introduce an original multidimensional method that combines preparative Centrifugal Partition Chromatography (CPC) to fractionate the hydrolysate coupled with Capillary Zone Electrophoresis (CZE) hyphenated online with mass spectrometry (MS). The method offers a green chemistry compliant analytical technique for the characterization of the peptides present in the CPC fractions, while an ELISA based assay allows the screening of the bioactive CPC fractions against a specific target: the Toll- like receptor 4 (TLR-4), a key activator of innate immune response against bacterial infections. Database search, and sequence alignments of the peptides identified by CZE-MS is implemented to guide bioactive peptide discovery.

Expanding PFAS detection in air using LC-HRMS-based non-target analysis

P11

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Non-target analysis (NTA) using high-resolution mass spectrometry (HRMS) is increasingly applied to extend the detection of per- and polyfluoroalkyl substances (PFAS) beyond conventional target lists, particularly in complex matrices such as air. In this study, an LC-HRMS-based NTA workflow was applied to 51 air samples. Particulate-phase compounds were collected on filters, while gas-phase compounds were captured using polyurethane foam (PUF) and XAD sorbents via active pumping. Passive deposition was collected in glass jars. Samples were obtained during 2024 monitoring campaigns at both industrial and background locations in Flanders, Belgium.

Data processing combined suspect screening with PFAS-specific prioritization strategies, including mass defect filtering and homologous series detection. Compound annotations were assigned according to the Schymanski confidence scale. Across all samples, only a limited fraction of PFAS-related features could be attributed to target compounds: on average, 13% were classified as confirmed PFAS (CL1), 14% as identified but previously untargeted PFAS (CL2–4), and 73% remained unknown (CL5). The large proportion of unknown features highlights both the complexity of PFAS contamination in air and the limitations of current target-based monitoring approaches.

In several samples where target analysis reported no detectable PFAS, the NTA workflow revealed a potential PFAS-related signal composed of unknown features. This demonstrates the ability of HRMS-based NTA to uncover previously unrecognized contamination and expand PFAS chemical space coverage. Moreover, inclusion of CL2 to CL5 features significantly altered PFAS fingerprints compared to target-only (CL1) compounds in several locations, proving an important tool for forensics purporsed.

The NTA approach enabled the detection of emerging PFAS classes, including perfluoroalkyl sulfonamide ethanols (FASEs) and fluorinated pesticides, as well as ultra-short-chain PFAS such as trifluoroacetic acid (TFA), perfluoropropanoic acid (PFPrA), and trifluoromethanesulfonic acid (TFMS). However, detection of highly polar and early-eluting compounds remained challenging due to chromatographic and data processing constraints, suggesting that their occurrence may be underestimated.

These findings highlight both the analytical power and current limitations of LC-HRMS-based PFAS NTA. While the approach increases detection coverage and supports feature prioritization, still a large fraction of signals remains structurally unknown. Moreover, expanding analytical coverage through complementary techniques (e.g., GC-HRMS, alternative LC modes) and improving data processing strategies will be essential to expand the characterization of the PFAS chemical space in atmospheric samples.

withdrawn

P12

An inorganic “non-targeted” characterization platform for comprehensive monitoring of elements in surface water

P13

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The concept of bioavailability influencing environmental risk from metals in Europe has gained regulatory and scientific support, but routine implementation remains a challenge due to the scarcity of physico-chemical data in national surface water monitoring networks for assessing metal complexation with abiotic ligands [1]. The focus blinders in our surface water monitoring networks should not transform into vision blinders, preventing us from considering broader insights and possibilities.

Thanks to advancements in analytical instruments and data processing capabilities, this study evaluated the feasibility of a comprehensive inorganic quantitative non-target analysis combined with geochemical modeling as holistic monitoring, i.e., a complete and comprehensive characterization of matrix and trace elements in 50 Flemish surface water samples. For this purpose, state-of-the-art inductively coupled plasma-tandem mass spectrometry (ICP-MS/MS) and Discrete Analyzer instrumentation were employed to perform quantitative analysis of (nearly) all elements in the periodic table. The obtained quantitative analysis data served as input for the PHREEQC geochemical model to calculate the chemical species of the elements and estimate the concentration of the free metal ion, a “target” for ecotoxicity [2]. For elements contributing to exceedances of the annual average quality standard (AA-QS) in Flemish waterways (e.g. Co, Zn), holistic monitoring can enhance understanding of their "(biological) availability" and ecological impact relative to environmental quality standards derived from chronic ecotoxicity data (NOEC or EC10). Different approaches to study the bioavailability of metal/metalloid species in freshwaters are compared, including the implementation of biotic ligand model-based approaches in risk assessment frameworks.

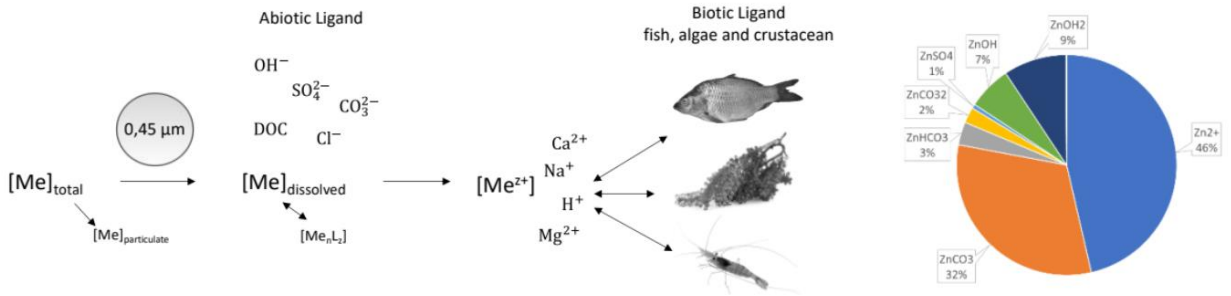

Figure 1. The Biotic Ligand Model (BLM) conceptual framework illustrating the abiotic speciation component and the biotic interactions; Output of a Zinc species distribution in a Flemish surface water (Bollaerdbeek, Ieper).

Examples of the added value of holistic monitoring, i.e. coupling of a non-target characterization platform with geochemical modeling, are given in terms of i) improved analysis quality, on the entire sample and not only on individual parameters, ii) Improved interpretation, not limited to the dissolved concentration per element but also assessing the concentration for each element species, iii) proactive policy on future contaminants by investigating elements that are currently not standardly monitored [3].

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Non-targeted screening of PFAS in food samples using LC-TIMS-HRMS

P14

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Per- and polyfluoroalkyl substances (PFAS) constitute a large and structurally diverse class of contaminants of increasing concern in food safety. Routine monitoring is generally based on targeted methods covering a limited number of well-characterized compounds, potentially overlooking emerging or less studied PFAS. In this context, non-target high-resolution mass spectrometry approaches offer new opportunities to broaden analytical scope and improve chemical coverage.

In this study, food samples were first analyzed by Sciensano using a validated targeted LC- MS method to quantify routinely monitored PFAS. The same extracts were subsequently investigated using a non-target liquid chromatography-trapped ion mobility-high resolution mass spectrometry (LC-TIMS-HRMS) workflow. Analyses were performed on a timsTOF Pro2 (Bruker) operating in PASEF mode. At this preliminary stage, data processing was largely performed manually. Kendrick Mass Defect analysis enabled the detection of several homologous series consistent with PFAS-related chemistries. In addition to the compounds identified by the targeted method, multiple additional features were observed, suggesting the presence of longer-chain PFAS and PFAS families not included in routine monitoring lists. Tentative annotations were supported by accurate mass measurements, retention time trends, collision cross section values derived from ion mobility, and characteristic fragmentation patterns, including specific neutral losses.

The integration of chromatographic separation, ion mobility information, and fragmentation were combined to strengthen confidence in structural hypotheses. Nevertheless, due to the lack of authentic reference standards and confirmatory MS/MS spectra for several features, the newly proposed identifications remain tentative and require further investigation.

These results highlight value of LC-TIMS-HRMS operating in PASEF mode for expanding
PFAS screening strategies in food matrices.

The research that yielded these results, was funded by the Belgian Federal Public Service Health, Food Chain Safety and Environment through the contract RT 23/07 PFASFORWARD.
Les recherches sous-jacentes à ces résultats ont reçu un financement du Service public fédéral belge Santé publique, Sécurité de la Chaîne alimentaire et Environnement au titre du contrat RT 23/07 PFASFORWARD.
Het onderzoek dat tot deze resultaten leidde, werd gesubsidieerd door de Belgische Federale Overheidsdienst Volksgezondheid, Veiligheid van de Voedselketen en Leefmilieu via het contract RT 23/07 PFASFORWARD.

Extreme ultraviolet photodissociation (XUVPD): coupling a benchtop mass spectrometer to a source of intense, high-energy photons

P15

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Ultraviolet photodissocation (UVPD) has proven to be a useful alternative fragmentation mechanism that provides additional insight into the structure of biomolecules. One drawback of the technique is that it relies on the use of laser- based systems with wavelengths of 266 nm, 213 nm and 192 nm being the most commonly uses. This is not ideal for two reasons. Firstly, at these wavelengths the fragmentation relies on the resonant excitation of high lying excited states, with fragmentation occurring during their relaxation. This is not ideas as if a biomolecule does not have a strong absorption in this region – or the absorption does not lead to diagnostic fragmentation – then UVPD is of limited use. The second drawback is the use of laser systems, which require both stringent safety requirements, alignment and thus are not simply turn-key solutions available to every laboratory. Here, we couple a high intensity helium lamp (Prevac UVS 40A2) to a Thermo QExactive+ bench-top mass spectrometer. The helium lamp is coupled directly to the vacuum chamber of the mass spectrometer, making it safer and with less alignment requirements. The much higher photon energies from the helium lamp can cause photoionisation processes to occur, which will open a variety of new fragmentation channels, and does not rely on absorption bands. This gives a wider variety of fragments on a wider variety of molecular ions. We show a comparison of the collision induced dissociation (CID) and XUVPD dissociation spectra for a series of small test ions, polysaccharides, peptides, proteins, lipids and oligonucleotides. Comparison of these spectra allow us to find differences in the fragmentation using XUVPD and discuss which biomolecular classes it can help provide additional structural information.

Exploring the conformational landscape of biomolecules: from standard proteins to the α-synuclein/aptamer complex using CIU and IM-MS

P16

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Introduction: Structural characterization of biomolecules is crucial for understanding structure-activity relationships. While NMR or CryoEM are efficient, they remain time-consuming. Ion Mobility-Mass Spectrometry (IM-MS) and Collision Induced Unfolding (CIU) offer rapid gas-phase alternatives for global shape analysis and conformational stability monitoring. However, correlating gas-phase "unfolding" (via soft ion-neutral collisions) with solution-phase thermal denaturation requires validation and careful investigation, which is the primary focus of this work.

Methods: Experiments were conducted on a modified Synapt G2-S (Waters) in non-denaturing conditions (ammonium acetate). Horse Myoglobin served as the initial benchmark. The study focuses on the Parkinson-related non-covalent complex between α-synuclein and the AS1 aptamer. Electrospray (ESI) and Nano-ESI parameters were optimized to preserve the complex in the gas phase. CIU heatmaps were generated by ramping collision voltages, monitoring the evolution of arrival time distributions (ATD) and Collision Cross Sections (CCS) to probe the energy landscape and resilience of the global shape of ions upon heating.

Main results: The CIU heatmap of myoglobin [M+9H]9+ ions validated the stability of the experimental setup. Crucially, the non-covalent complex formed by the intrinsically disordered α-synuclein and the structured nucleic acid aptamer was successfully mass-selected. Its CIU fingerprint revealed a unique coexistence of two distinct metastable conformer populations at higher activation energies. Comparative CIU analysis of the isolated aptamer suggests that the structural rearrangement of the nucleic acid moiety might drive the complex's unfolding. Ongoing work aims to correlate these gas-phase thermal denaturation patterns with solution-phase data (e.g., DSF) to validate CIU for screening therapeutic ligands. Additionally, this method allows simultaneous monitoring of complex survival yield, unfolding, dissociation, and fragmentation channels.

Monitoring changes in the levels of newly synthesized proteins in response to Nutlin-3 treatment

P17

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Developing methodological approaches for discovering novel pathways is a crucial aspect in the life science research. Biological pathways are regulated—in higher eukaryotes—by a vast diversity of linear peptide motifs that mediate combinatorial specificity in signal transduction pathways. Indeed, the E3 ubiquitin ligase component (MDM2) is a protein which interacts with target proteins containing linear motifs such as p53. MDM2 is a RING-domain E3 ubiquitin ligase and a proto-oncogene which regulates the stability of its client proteins via catalysing the transfer of ubiquitin to respective substrates. The most well-known client protein ubiquitinated by MDM2 is p53, the transcription factor regulating expression of proapoptotic and growth-arresting genes, therefore acting as a tumour suppressor. Drug leads, including Nutlin-3, that bind to the MDM2 hydrophobic pocket mimic p53, hence releasing p53 from MDM2 control and this can lead to cell death. Neverthless, these drug leads act allosterically, having agonist effects on MDM2's functions and there are other proteins whose steady state levels can be altered by Nutlin-3. These changes are by definition MDM2-dependent since Nutlin-3 can interact with MDM2, however we cannot rule out MDM4 dependencies. As cell density can alter the proliferation state of cell populations, we examined the impact of Nutlin-3 on levels of newly synthesized proteins using pulse-SILAC mass spectrometry. The data demonstrate that at differing cell densities or population-wide proliferation rates, different newly synthesized proteins dominant the proteome landscape in a Nutlin-3 dependent manner. Our findings further confirm that the cell state in a population of cells can in turn alter the MDM2 signalling landscape. Therefore, this methodological approach may form a blueprint for biomarker discovery which can identify and detect changes in the synthesis rate of proteins in drug-treated cell types with more direct clinical value.

Optimization of LC-HRMS non-target fata processing for PFAS: improving feature prioritization and annotation confidence

P18

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Non-target analysis (NTA) of per- and polyfluoroalkyl substances (PFAS) using LC-HRMS requires robust and reproducible data processing workflows to efficiently reduce complex datasets and prioritize relevant features. In this work, an in-house NTA-PFAS data processing workflow was developed based on the Thermo Scientific Compound Discoverer™ PFAS workflow, integrating literature-derived strategies and tailored modifications to improve selectivity, sensitivity, and annotation confidence.

Raw LC-HRMS data (full MS/dd-MS^2) were processed through a multi-step workflow including feature detection, compound grouping, annotation, and prioritization. Key adaptations were implemented at different processing nodes. Feature detection parameters were optimized by increasing the minimum peak intensity threshold (2000) and limiting MS^2 trees (max. 3) to reduce noise while retaining relevant PFAS features. Compound assembly was restricted to $[M–H]^-$ ions, excluding fragments during grouping to improve consistency of feature annotation.

Data reduction and prioritization relied on a combination of orthogonal PFAS-specific filters. Fragmentation-based screening was enhanced using mzCloud and a curated inhouse mzVault spectral library, with adjusted scoring thresholds and prioritization of in-house spectral data. A neutral loss search node was incorporated to improve detection of PFAS- related transformation patterns. Compound class scoring thresholds were increased ($S/N \geq 3$) to enhance selectivity toward PFAS-specific fragmentation signatures. Additionally, a PFAS- specific fragment list was implemented to support class-based annotation.

To further refine candidate selection, mass defect filtering and Kendrick mass defect analysis were applied, exploiting characteristic fluorine-related patterns and homologous series behavior, as described in the original workflow. An orthogonal discrimination approach based on normalized mass (m/C) and mass defect (MD/C) was used to identify PFAS clusters within HRMS feature space.

An automated confidence level assignment system based on the Schymanski scale, adapted for PFAS (Charbonnet et al., 2022), was implemented to standardize identification reporting. Workflow performance showed sensitivity of 87% and selectivity of 76% (Jacob *et al.*, 2021), and a workflow-specific LOQ was determined at 5ppb for compounds to be able to be assigned as confidence level 1, where MS/MS data is needed.

Overall, the developed workflow enables efficient data reduction, robust PFAS prioritization, and harmonized confidence annotation in LC-HRMS NTA datasets. The integration of multiple orthogonal filtering strategies and tailored parameter optimization significantly improves the balance between sensitivity and selectivity, supporting more reliable characterization of PFAS in complex environmental samples.

Coupling of ion mobility to MALDI mass spectrometry for non-targeted imaging of PFAS

P19

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Per- and polyfluoroalkyl substances (PFAS) comprise a structurally diverse class of persistent environmental contaminants whose spatial distribution in biological matrices remains poorly understood. While matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) offers unique opportunities for in situ PFAS mapping, non-targeted detection remains challenging due to low ionization efficiency, extensive in-source fragmentation, and spectral interferences from complex biological backgrounds. This work investigates the coupling of trapped ion mobility spectrometry (TIMS) with MALDI-MS as a strategy to enhance the non-targeted imaging of PFAS through mobility-based signal filtering.

A dual-matrix system based on norharmane (NoH) and 1,5-diaminonaphthalene (DAN) was optimized for PFAS detection. Kendrick mass defect (KMD) analysis on a CF_2 basis enabled systematic characterization of PFAS-related fragmentation pathways. Perfluoroalkyl carboxylic acids (PFCAs) exhibited extensive fragmentation, predominantly yielding decarboxylated and defluorinated ions, while perfluoroalkyl sulfonic acids (PFSAs) showed greater stability.

The optimized workflow was evaluated through imaging experiments on mouse brain sections spiked with a PFAS mixture and analyzed using both MALDI-TOF-MS and MALDI-TIMS-TOF-MS platforms. Conventional MALDI imaging enabled clear visualization of PFSA distributions, whereas PFCA detection remained limited by low signal intensity and fragmentation. The incorporation of TIMS significantly improved selectivity by separating PFAS ions from matrix-related and endogenous interferences based on their characteristic mobility-to-mass trends. Distinct PFSA and PFCA mobility trendlines were observed in $1/K_0$ versus *m/z* heatmaps, enabling class-specific signal filtering and reconstruction of PFAS images with improved contrast and signal-to-noise ratios.

These preliminary results demonstrate that coupling ion mobility to MALDI-MSI substantially enhances non-targeted PFAS detection and discrimination in complex biological matrices. By combining optimized matrix deposition, KMD-assisted annotation, and mobility filtering, this workflow establishes a robust foundation for spatially resolved investigation of PFAS accumulation in environmental and biological model systems, including Daphnia magna, and opens new perspectives for the non- targeted imaging of emerging fluorinated contaminants.

A case study of aptamer-target complexes characterization by frontal analysis capillary electrophoresis and ion mobility mass spectrometry

P20

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In recent years, oligonucleotide-based therapeutics—particularly aptamers that are single-stranded oligonucleotides that recognize specific molecular targets with high affinity and selectivity, emerged as highly valuable tools with applications ranging from biosensing to therapeutics. However, designing and fully characterizing aptamers remain challenging, as their key properties depend on their higher-order structures (HOS) and thermodynamic behavior and when ligand recognition involves coupled equilibria with coordinating cations. As a result, in the context of aptamer development, where candidates often need to be compared and refined, the development of a strategy combining rapid comparative tools with precise reference measurements is highly anticipated. In this study, we investigated the aptamer OTC5, which binds the antibiotic oxytetracycline (Oxy) only in the presence of Mg^{2+} .

In this work, we propose a combined methodology of Mass spectrometry (MS), ion mobility- collision induced dissociation (CID) and collision induced unfolding (CIU), together with frontal analysis capillary electrophoresis (FACE), to investigate the OTC5-Mg^{2+} -Oxy ternary complex. FACE provided a robust apparent affinity constant, of 1.45 µM in solution and revealed that nearly all the OTC5 population is binding-competent under our conditions. MS titration yielded a higher apparent , reflecting its limitations inherent to response factors and gas-phase stability as evidenced by CID. However, CIU experiments provided conformational information on the complex, evidencing the further stabilization of the complex upon oxytetracycline binding. Finally, preliminary competitive binding experiments showed that MS reports relative affinities between OTC5 and structurally related tetracyclines, supporting its use as a rapid comparative tool. All together, these results illustrate the complementarity of solution and gas-phases analysis for such aptamer-target complex characterization.

Parallel targeted and untargeted metabolite analysis of mouse plasma samples using a benchtop multi-reflecting time of flight mass spectrometer

P21

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Metabolomic profiling of biological matrices can be targeted (quantifying known metabolites) or untargeted (identifying unknown molecules). Traditionally, these analyses are performed separately on different MS platforms due to their specific performance attributes. However, advancements in instrument sensitivity and scanning capabilities now allow both approaches on a single platform, reducing resource and sample volume requirements.

The Xevo MRT's fast-scanning capabilities enable the targeting of 65 amino acids and internal standards while generating high-quality untargeted data for biomarker discovery. Of these, 20 amino acids were fully quantifiable against stable isotopically labelled standards (SILS), and the remaining 40 were quantified against the SILS based on retention time. Amino acids were quantified over a 5–1000 µM range, with calibration curve linearity ($R^2 > 0.99$) and 67% of QC standards within 15% of the nominal concentration.

Simultaneously, untargeted data were processed using MZmine software for peak picking and normalization. Statistical analysis, including PCA and OPLS-DA, highlighted significant features between sample groups. Combining both acquisition approaches in a single analysis showed minimal compromise on data quality, maintaining high-resolution accurate mass untargeted analysis data.

Determination of pesticide residues in cucumber using GC-MS/MS with APGC after extraction and clean up using QuEChERS

P22

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Reliable analytical methods are needed for detection, quantification, and identification of hundreds of pesticide residues in many different commodities. This application note describes the development and validation of a comprehensive method based on GC–MS/MS for the determination of over 200 pesticides. Extracts of cucumber were prepared using the CEN version of QuEChERS, including a dispersive solid-phase extraction (dSPE) step followed by determination with GC-MS/MS. The use of GC-MS/MS utilizing atmospheric pressure ionization (APGC) has been shown to offer significant improvements in performance over electron ionization (EI) for pesticide residue analysis, in terms of selectivity, specificity, and speed of analysis. The extremely high sensitivity of the APGC Xevo™ TQ Absolute XR system was demonstrated with reliable detection for all the analytes at concentrations as low as 0.001 mg/kg, even when injection volume was limited to 1µL. The method was successfully validated in cucumber using the SANTE guidelines document. The results from analysis of the spikes @ 0.001 mg/kg showed that 94 % and 99 % of the analytes were within the required tolerances for recovery and repeatability, respectively. The method is considered sensitive, specific, accurate, and suitable for the determination of residues of a wide range of GC-amenable pesticides in agricultural commodities for checking compliance with MRLs and has the potential for determination at much lower concentrations.

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Adaptation of large panels of per- and polyfluoronated alkyl substances (PFAS) for routine analysis in drinking and environmental waters by direct injection using UHPLC-MS/MS

P23

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Per- and Polyfluoroalkyl Substances (PFAS), are extremely persistent in the environment. Their production and use have resulted in severe contamination of soil, water and food. To protect public health, advisory and regulatory limits continue to be created and updated. Consequently, routine PFAS analysis has become challenging as not only low detection limits are required, but extensive compound coverage is a prerequisite nowadays.

The purpose of this work was to demonstrate a direct injection UHPLC-MS/MS method for the ultra-low-level determination of multiple PFAS compounds in drinking and environmental waters. The method performance study was completed on an ACQUITY™ Premier System with a Xevo™ TQ Absolute XR mass spectrometer and UniSpray™ Ion source. Samples were prepared by dilution with an acidified organic solution containing internal standards directly into an autosampler vial.

A method validation study was carried out on 2 common drinking water and 2 surface water matrices. The method performance was assessed using 3 spike levels at 1, 5, and 10 ng/L for all analytes, with 6 replicates at each level. Average method performance for trueness, repeatability, linearity, and sensitivity was assessed through inter and intra-laboratory studies.

Improvements in both the analytical and isolator column technologies demonstrated in this work, as well as enhancements in negative ion sensitivity from the Xevo™ TQ Absolute XR Mass Spectrometer are helping to support ongoing efforts in PFAS analysis. This allows for easier, more robust, and accurate options as PFAS analysis continues into the future.

Characterization of phosphorothioate (PS) diastereomers in a phosphorothioated antisense oligonucleotide using ion mobility mass spectrometry

P24

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Introduction

Synthetic oligonucleotides are promising therapeutics that can regulate gene expression to treat and prevent diseases. To enhance their performance and stability, they are often modified with phosphorothioate (PS) linkages along their phosphodiester backbone. This modification introduces a chiral center, resulting in each PS linker having either Sp or Rp configuration, leading to the formation of (2^n) diastereomers. This can result in thousands of isomers in a sample, making analytical characterization of these challenging. A method is presented to characterize these diastereomer ratios along the oligonucleotide backbone utilizing the SELECT SERIES™ Cyclic™ IMS system

Methods

Initial experiments are conducted via direct infusion, utilizing generic ion mobility separation times in ESI-MSMS and ESI- MS^E to sequence the oligonucleotide with the waters_connect™ CONFIRM Sequence application. This software facilitates sequence creation, automatic fragmentation predictions, and spectral assignment. Once sequenced, we assess the mobility of monomer fragment ions. This assessment helps determine the types of fragment ions (e.g., a, b, c, d, w, x, y, or z), which exhibit mobility separations characteristic of phosphorothioate chirality, rather than separations based on other structural features. Once identified, the relevant fragments are targeted for multipass experiments using the Cyclic device to enhance ion mobility separation and resolution compared to single-pass experiments.

Preliminary Data

A synthetic ASO type oligonucleotide was chosen to determine the capabilities of the Cyclic Ion mobility system for phosphorothioate analysis and the CONFIRM Sequence software tool with regards to sequence confirmation.
ESI- MS/MS and MS^E spectra were acquired and the oligonucleotide fragment ions were compared against the predicted ions generated according to the CID fragmentation rules of oligonucleotides. The CONFIRM sequence software is able to calculate the expected CID fragmentation for the target sequence. Data processing is then performed and a comprehensive review of the data is presented. The software was able to provide 100% coverage of the target oligonucleotide sequence based on the fragmentation data. Once relevant fragments were determined, use of the Cyclic ion mobility system's unique pre IMS fragmentation capabilities allowed separation and characterisation of these fragment ions for investigation into terminal and internal dimer and trimer diastereomer fragment ion conformers which provided a novel approach allowing characterization of each linker along the length of the phosphorothioated oligonucleotide backbone. These characteristic fragment ions correspond to expected isomer profiles providing the potential to investigate stereochemistry of the phosphorothioate linkers and demonstrate the capability for phosphorothioate isomer quantification across sample batches. Multipass experiments utilising the Cyclic device for increased separation times provided improved separation of oligonucleotide fragment ions compared to single pass experiments. Additionally, the systems unique trapping and slicing capabilities with subsequent fragmentation allows characterisation of a specific linker where the mass/sequence of the 1^{st} or 2^{nd} generation fragment ion is non-unique.

Novel Aspect

Oligonucleotide Cyclic ion mobility characterization of diastereoisomer conformers including internal fragments to allow phosphorothioate backbone characterization.